EXAMINING COMORBIDITY, PRENATAL RISK FACTORS, AND THE GENETIC DETERMINANTS OF COGNITIVE DEVELOPMENTAL TRAJECTORIES FOR INSIGHTS INTO AUTISM SPECTRUM DISORDER

by

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ABSTRACT

Background: Autism spectrum disorder (ASD) is a heritable, heterogeneous neurodevelopmental disorder affecting social communication and motor stereotypies. Comorbid mental and physical conditions, perinatal risk factors, genetic risk factors, and early developmental trajectories of communication, social, and motor abilities are important sources of phenotypic and etiologic heterogeneity of ASD and other neurodevelopmental disorders. **Methods:** Data from the National Comorbidity Survey Adolescent Supplement (NCS-A) were used to characterize mental and physical comorbidity and parent report of perinatal risk factors among ASD and learning disorder (LD) in a nationally representative sample of US adolescents. Two enriched risk infant cohorts, the Early Autism Risk Longitudinal Investigation (EARLI) and the Infant Brain Imaging Study (IBIS) were used to identify common ASD-related genetic variation associated with differential trajectories of cognitive and motor development. **Results:** In the NCS-A, both ASD and LD adolescents were more likely to have gastrointestinal problems, epilepsy/seizures, and attention-deficit/hyperactivity disorder (ADHD), while ASD, but not LD, had increased risk for allergies, acne, heart problems, and sleep disturbances. Prenatal exposure to alcohol, maternal urinary tract infections, slow heartbeat, and respiratory distress at birth were associated with both ASD and LD. Persistent proteinuria and neonatal complications were associated with ASD but not LD. In the EARLI/IBIS combined cohort, three latent classes were identified and labeled "normative", "intermediate", and "declining" based on longitudinal patterns of the Mullen Scales of Early Learning. ASD polygenic risk score was marginally associated with the "declining" class when compared to the "normative" class and was significantly

associated with ASD diagnosis. Testing SNPs in ASD candidate genes revealed associations of suggestive significance ($p < 1x10^{-3}$) at chr3:2928197 in *CNTN4* and rs10265509 in *CNTNAP2* on chromosome 7, comparing the "intermediate" to the "normative" class. **Conclusions:** ASD and LD appear to share many comorbid conditions and pre-/perinatal risk factors. Gastrointestinal problems, epilepsy/seizures, ADHD, and insults during the pre-/perinatal period may be associated with neurodevelopment broadly. These findings may have important implications for clinical evaluation of adolescents with neurodevelopmental disorders. Genes coding for proteins involved in cell-cell interactions and neuronal migration in the developing nervous system may be specifically related to early cognitive developmental trajectory.

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INTRODUCTION AND REVIEW OF LITERATURE

1.1. Overview

Autism spectrum disorder (ASD) is an early onset neurodevelopmental disorder (NDD) characterized by social and communication deficits and restrictive and repetitive interests or behaviors and results in varying degrees of lifelong functional impairment (American Psychiatric Association, 2013). ASD is a phenotypically heterogeneous disorder, presenting with varying degree of severity, symptomatology, onset trajectory, and comorbidity profiles. ASD is also etiologically heterogeneous, with strong evidence that both genetic and environmental risk factors each play a significant role, either independently or interactively (Gaugler et al., 2014; Hallmayer et al., 2011; Sandin et al., 2014). It is likely that multiple combinations of risk factors, genetic and environmental, contribute to ASD susceptibility. Additionally, both genetic and environmental risk factors associated with ASD may contribute to neurodevelopment broadly (Pettersson, Anckarsäter, Gillberg, & Lichtenstein, 2013; Schieve, Clayton, Durkin, Wingate, & Drews-Botsch, 2015). Here, we examined patterns of physical and mental comorbidity and perinatal environmental risk factors for specificity to ASD among a population-based sample of US adolescents. We then examined the relationship between ASD genetic risk variants and early cognitive developmental trajectory among infants enrolled in an enriched risk cohort.

1.2. Organization

The current chapter (Chapter 1) introduces the motivation and goals of this dissertation and provides a review of the existing literature. We begin with a brief history of Autism as a diagnostic entity and then describe the current diagnostic criteria and procedures. Then, we will describe the current burden and demographic correlates of ASD. Next, we will describe clinical correlates of ASD, including both physical and mental comorbidity, as well as its relationship to other neurodevelopmental disorders. This will be followed by what is known on the etiology of ASD, consisting of a review of evidence for both genetic and environmental risk factors. Methodological challenges to studying ASD epidemiologically will then be discussed. This will be followed by a review of ASD onset heterogeneity, and arguments for both combining *across* and splitting *within* diagnostic boundaries in etiologic research. Finally, we state the specific aims of this work.

Chapter 2 presents an analysis of prevalence and risk for various physical and mental health problems among adolescents with ASD and learning disorder (LD) from the National Comorbidity Survey Adolescent supplement (NCS-A). Chapter 3 presents a similar analysis of prevalence and risk for ASD and LD in the presence of various pre- and perinatal risk factors in the NCS-A. Chapter 4 presents an association analysis between purported ASD-related genetic variants associated with latent trajectories of early cognitive and motor development among infant siblings of children with ASD in the Early Longitudinal Risk Investigation (EARLI) and the Infant Brian Imaging Study (IBIS). Chapter 5 concludes this dissertation with a review of findings from the three manuscripts, intentions for future research, and public health significance of this work.

1.3. Background on autism spectrum disorder

The term "autism" was originally coined in 1908 by Swiss psychiatrist Eugen Bleuler, and was used it to describe severely withdrawn schizophrenic patients who had difficulty engaging with the outside world (Crespi, 2010). However, in the 1940's the term was redefined by Leo Kanner and Hans Asperger, who independently began to describe children with social difficulties, sensitivity to various stimuli, and general difficulty adapting to change in daily routines, with varying degrees of linguistic problems. Over the next sixty years, the name and diagnostic criteria would continue to change over time. From 1994 to 2013, the Diagnostic and Statistical Manual of Mental Disorders (DSM) defined ASD as a group of three separate disorders that included autistic disorder, Asperger's syndrome, and pervasive developmental disorder – not otherwise specified (PDD-NOS) (American Psychiatric Association, 2000). These criteria were in effect at the time of data collection for the NCS-A survey and were used for final evaluation of infants in the IBIS cohort. Therefore this dissertation will report on ASD as defined by DSM-IV. However, in 2013, the DSM-5 was published and made some changes to the way we currently diagnose ASD. It was re-conceptualized into one disorder that may vary in severity on a spectrum. The diagnostic criteria are presence of A) deficits in social communication and social interaction in multiple contexts and B) restricted repetitive behaviors, interests, or activities. Examples of social and communication deficits include failure to initiate or respond to social interactions, difficulty making eye contact or understanding use of gestures, or absence of interest in peers. Examples of restricted repetitive behaviors include repetitive movements or speech, inflexibility with change in routine, intense fixation on interests, and increased or

decreased sensitivity to sensory input. Symptoms from both domains must be present with onset in early development and cause significant impairment in functioning (American Psychiatric Association, 2013). The purpose for diagnostic changes was to enable earlier diagnosis and to streamline diagnoses across clinical settings. Changes made from DSM-IV are relatively minor and should result in similar prevalence estimates and minimal change in diagnostic status (Huerta, Bishop, Duncan, Hus, & Lord, 2012; Maenner et al., 2014).

While some known genetic syndromes carry increased likelihood of presenting ASD related symptoms (Miller et al., 2010), there are no known biomarkers specifically for ASD (G. M. Anderson, 2015). Therefore, diagnosis must be made by direct observational assessment by a clinician. Clinicians will often use the Autism Diagnostic Observation Scale (ADOS) (Lord et al., 1989) and Autism Diagnostic Interview- revised (ADI-R) (Lord, Rutter, & Le Couteur, 1994) to aid in diagnosis and assessment of individuals with ASD. The ADOS is used to assess social and communication behaviors related to autism symptoms by direct interaction with the subject. The ADI-R is a structured interview conducted with parents or caregivers of a child referred for ASD evaluation. The ADI-R evaluates language and communication skills, reciprocal social interactions, and restricted and repetitive behaviors/interests. The best way to obtain an accurate ASD diagnosis is through evaluation by experienced clinicians aided by diagnostic instruments such as the ADOS and ADI-R (Lord et al., 2006).

1.4. Descriptive epidemiology and public heath burden of autism spectrum disorder

According the Centers for Disease Control, the incidence of ASD is increasing over time (Boyle et al., 2011). US estimates from educational and health records indicated that 1 in 68 eight-year-old children met criteria for ASD in 2012 (Christensen et al., 2016) and 2010 (CDC, 2014), compared to 1 in 88 in 2008 (CDC, 2012) and 1 in 110 in 2006 (CDC, 2009). The most recent national estimates indicate significant differences in distribution by gender, race/ethnicity, and region (Christensen et al., 2016). Prevalence among boys was nearly 4.5 times greater than girls (23.6 compared to 5.3 per 1,000 eight-year-olds). Prevalence of identified ASD was significantly higher among non-Hispanic white children compared to Hispanic and non-Hispanic black children. The authors suggest this disparity is likely due to lack of treatment and service availability among Hispanic and black populations rather than a lower actual prevalence. The data also indicated that Hispanic children were more likely to be identified later than non-Hispanic white and non-Hispanic black children. There were also regional disparities in ASD prevalence, ranging from 8.2 to 24.6 per 1,000 eight-year-old children. Regions where research sites had access to both school and health records had significantly higher prevalence of identified ASD than sites where only health records were reviewed. This suggests the educational system plays a significant role in evaluating and providing services to affected children.

ASD adds significant financial burden to affected families and communities. The estimated cost of supporting an individual with ASD over the lifespan is $2.4 million US with co-occurring intellectual disability (ID) and $1.4 million US for ASD without ID (Buescher, Cidav, Knapp, & Mandell, 2014). During childhood, the majority of cost is attributed to special education needs and parental lost wages. In adulthood, the majority

of cost is attributed to residential and assisted living needs and lost personal wages (Buescher et al., 2014). Having a child with ASD limits earning potential and incurs extra costs, the combination of which creates a substantial financial burden to families (Cidav, Marcus, & Mandell, 2012). Primary caregivers of children with ASD are likely to earn substantially less (Cidav et al., 2012), experience instability in employment, and are less likely to partake in leisure activities (Hodgetts, McConnell, Zwaigenbaum, & Nicholas, 2014). In addition, family members of affected children are more likely to experience divorce, higher levels of distress, lower well-being, and higher rates of mental and physical health problems than family members of typically developing children and children with other developmental disorders (Karst & Van Hecke, 2012; Keenan, Newman, Gray, & Rinehart, 2016).

Adults with ASD experience decreased earning potential and increased living and medical costs (Buescher et al., 2014) and are more likely to be unemployed, underemployed, or employed at jobs not well suited to their abilities, resulting in low employment retainment and financial insecurity (Roux et al., 2013; Shattuck et al., 2012). Even high functioning ASD adults, despite capacity and willingness to work, face challenges in the workplace related to lack of understanding, and lack of support (Baldwin, Costley, & Warren, 2014). Additionally, adults with ASD are less likely to live independently (K. A. Anderson, Shattuck, Cooper, Roux, & Wagner, 2014), experience more social isolation, higher rates of mental and physical comorbidity, and will have decreased life expectancy compared to adults without ASD (Perkins & Berkman, 2012).

1.5. Clinical correlates of autism spectrum disorder

1.5.1. Overview of autism spectrum disorder comorbidity

Children with ASD are more likely to experience an array of comorbid physical and mental health problems compared to typically developing children (Gurney, McPheeters, & Davis, 2006; Schieve et al., 2012). A school-based study found that over 70% of children identified with ASD had at least one comorbid condition (Memari, Ziaee, Mirfazeli, & Kordi, 2012). Cost of medical treatment for ASD-affected individuals significantly increases when they reach adulthood (Buescher et al., 2014). This section will provide background on the prevalence and shared risk factor s of other neurodevelopmental disorders that frequently co-occur with ASD, focusing on intellectual disability, learning disorder, and attention deficit hyperactivity disorder. Then evidence for the burden of various physical and mental health conditions among those with ASD will be reviewed.

1.5.2. Comorbid neurodevelopmental disorders

Neurodevelopmental disorders (NDDs) are a group of disorders characterized by impairments in cognition, communication, learning, motor skills, and/or daily functioning that typically emerge before adolescence and persist throughout the lifespan (American Psychiatric Association, 2013). ASD, intellectual disability (ID), attention deficit hyperactivity disorder (ADHD), and learning disorder (LD) are categorized as NDDs in the DSM5. NDDs often co-occur with one another, and current research suggests

potential commonality in both genetic and environmental risk factors (Lichtenstein, Carlstrom, Rastam, Gillberg, & Anckarsater, 2010; Pettersson et al., 2013; Schieve et al., 2015).

ID is characterized by limitations in both intellectual capacity (learning, reasoning, and problem solving) and adaptive functioning (conceptual, social and practical skills). IQ scores below 70 on standardized testing instruments generally indicate an ID. The overall prevalence of ID is between approximately 1-2% (Bhasin, Brocksen, Avchen, & Van Naarden Braun, 2006; Bourke, de Klerk, Smith, & Leonard, 2016; Boyle et al., 2011) while the prevalence of ID among those with ASD is estimated to be between 30% and 60% (CDC, 2007, 2014). There are many causes of ID, including genetic syndromes, environmental exposures, and brain injury, but the limited intellectual and adaptive functioning may result from common risk factors that also cause ASD or from symptoms of ASD itself (Hu, Chahrour, & Walsh, 2014).

LD is characterized by impairments in understanding, remembering, and processing new information. While these difficulties are likely present in early developmental years, LD is typically not recognized and diagnosed until a child enters school, where they tend to have difficulty in listening/paying attention, speaking, reading, writing, and/or math. Children with LD perform lower than expected on one or more of these areas of learning, but unlike ID, have IQ scores within the normal range (American Psychiatric Association, 2013). The prevalence of LD in the general population is approximately 10% (Altarac & Saroha, 2007). LD in the context of ASD is far less studied in comparison to comorbid ID, but prevalence estimates of LD among ASD from a national parent-report survey is estimated to be nearly 80% (Schieve et al., 2012).

Children with chronic health conditions are more likely to be affected by LD than healthy peers (Schulte, 2015). Several comorbid conditions (Schieve et al., 2012) and risk factors (Schieve et al., 2016) for ASD have also been reported in LD.

ADHD is a common NDD that affects approximately 3.4% of children and adolescents worldwide (Polanczyk, Salum, Sugaya, Caye, & Rohde, 2015) and is characterized by persistent problems with inattention, hyperactivity, and impulsivity that affect daily functioning. Symptoms are often identified in the school setting, but persist throughout adulthood(American Psychiatric Association, 2013). Although a concurrent diagnosis of ADHD and ASD was not possible under DSM guidelines until the recent release of DSM5, clinicians and parents have long noticed ADHD traits in children with ASD (Matson, Rieske, & Williams, 2013). Prevalence estimates of ADHD among ASD range from 40 to 50% in both clinically referred and population samples (Gurney et al., 2006; Matson et al., 2013; Schieve et al., 2012). Similar to ID and LD, ADHD has been linked to several comorbid physical conditions (allergy, asthma, epilepsy, sleep disturbances), perinatal, and genetic risk factors that we also see in ASD (Matson & Goldin, 2013; Nikolac Perkovic et al., 2014; Rommelse, Franke, Geurts, Hartman, & Buitelaar, 2010).

1.5.3. Comorbid physical conditions

Individuals with ASD reportedly experience chronic physical health conditions at a higher rate compared to their typically developing peers (Gurney et al., 2006; Schieve et al., 2012). The most studied comorbid physical conditions are epilepsy, gastrointestinal disorders, sleep disturbances, and allergic/autoimmune conditions; but

these studies tend to examine one comorbid condition at a time from clinically ascertained samples (Matson & Goldin, 2013). The reported prevalence of any GI disorder, including diarrhea, constipation, and abdominal pain, in ASD ranges from 9% to 90%, depending on the study design (Coury et al., 2012) and an estimated 4.5 times greater odds of having a GI disorder compared non ASD individuals(McElhanon, McCracken, Karpen, & Sharp, 2014). Approximately 50 to 80% of children with ASD are reported to have marked sleep disturbances compared to 9 to 50% of typically developing children. This is an increased rate in comparison to other developmental disorders (Reynolds & Malow, 2011) and is correlated with more cognitive and adaptive behavioral problems in among ASD children (Taylor, Schreck, & Mulick, 2012). The large range of prevalence estimates of epilepsy (5 to 46%) likely results from differences in sample characteristics (Spence & Schneider, 2009; Tuchman & Rapin, 2002) as others have shown that epilepsy is associated with both comorbid ID and gender, where the male to female ratio of affectedness narrows from 3.5:1 to 2:1 when restricting to those with comorbid epilepsy (Amiet et al., 2008). Allergy and other diseases involving mast cell activation are reported at higher frequency in ASD cases compared to controls and is thought to possibly create an inflammatory response that releases neurotoxic molecules (Angelidou et al., 2011; Theoharides, 2013).

Children with comorbid conditions tend to have higher ASD symptom severity than those without comorbid conditions (Memari et al., 2012). Proper identification of comorbid physical conditions may lead to treatment that can substantially improve quality of life and behavioral symptoms as well as potentially inform etiologically meaningful subtypes of ASD (Bauman, 2010). A recent analysis of electronic health

records studied patterns of comorbid medical conditions over time suggests that these comorbidities cluster distinctly and may indicate distinct etiologies (Doshi-Velez, Ge, & Kohane, 2014).

1.5.4. Comorbid mental disorders

A greater number of studies have examined psychiatric, or mental, comorbidity of ASD compared to physical comorbidity, with a greater proportion of studies examining more than one mental health disorder simultaneously; however, the vast majority of these studies are on ADHD, anxiety, and general psychopathology (Magiati et al., 2016; Matson & Goldin, 2013; Vasa & Mazurek, 2015). Limited research exists on the full range of mental health problems, including depression, bipolar disorder, eating disorders, and psychosis (Matson & Goldin, 2013). A sample of clinically referred adults found that those with ASD had nearly twice as many psychiatric problems, including major depressive disorder, and multiple anxiety disorders, compared clinically referred adults without ASD. Additionally, ASD adults experienced higher levels of functional impairment and a greater need for therapeutic and pharmacologic treatment (Joshi et al., 2013). Parents of children with ASD who participated in the National Survey of Children's Health reported high rates of anxiety and depression in their children (McPheeters, Davis, Navarre, & Scott, 2011), but we do not currently have population-based estimates of mental disorders in children or adolescents with ASD that were obtained from direct assessment of the affected individual.

1.6. Etiology of autism spectrum disorder

1.6.1. Overview of etiology

While the exact cause of ASD is unknown, research suggests that both genetic and environmental factors play a role in ASD etiology. Here, we will review the current state of the literature describing evidence supporting a polygenic nature of ASD risk. First we will discuss family-based heritability studies, then evidence for the contribution of common variants, followed by rare genetic variants, including inherited and *de novo* point mutations and structural variants. We will then discuss epigenetic changes that may provide a mechanism for gene-by-environment interaction effects that contribute to ASD risk. Finally, we review evidence supporting a hypothesis for early-life (preconception through perinatal periods) non-genetic (environmental) risk factors contributing to ASD risk, as previous work on neuroanatomic and serologic studies have suggested that ASD pathophysiology begins *in utero*, thus, the pre-/perinatal period as a critical window for exposure to potential etiologic risk factors (Bailey et al., 1998; Bauman & Kemper, 2005; Newschaffer, Fallin, & Lee, 2002; Rodier, 2002; Rodier, Ingram, Tisdale, Nelson, & Romano, 1996).

1.6.2. Genetic risk factors

Family heritability and relative recurrence risk

While early twin studies reported genetic liability of estimates at approximately 90%, recent family study research suggests that approximately half of the phenotypic liability for ASD can be explained by genetic factors (Gaugler et al., 2014). A comparison of dizygotic and monozygotic twins found an ASD probandwise concordance rate of .77 for monozygotic .31 for dizygotic twins, suggesting a genetic heritability

component of 38% for ASD (Hallmayer et al., 2011). Among families from a population-based cohort from Swedish national register data, relative recurrence risk was estimated at 153 for monozygotic twins, 8.2 for dizygotic twins, 10.3 for full siblings, and 2.0 for cousins (Sandin et al., 2014). The same study estimated the overall heritability for ASD to be approximately 50% (Sandin et al., 2014). An enriched risk cohort of infant siblings of children with ASD found that nearly 19% of siblings were diagnosed with ASD by 3 years of age, suggesting a nearly 20 fold risk compared to the general population (Ozonoff et al., 2011). More recently, a population-based twin sample from the UK estimated heritability of ASD and ASD-related traits to be between 56 and 95%, with higher estimates for stricter diagnostic criteria (Colvert et al., 2015).

Common genetic variation

Recent molecular genetic studies have suggested that the majority of genetic liability for ASD is the results of many common variants acting additively. Studies examining additive genetic effects have estimated that up to 50% of phenotypic liability in ASD is due to common genetic variation , while rare *de novo* variants, while they contribute substantially to individual liability, account for only about 2.6% of overall liability variance in the population (Gaugler et al., 2014; Klei et al., 2012; S. H. Lee et al., 2013).

However, genome-wide association (GWA) studies aiming to identify locus specific common variants have had limited success. Six GWA studies have been published to date, where only a few SNPs from these studies have reached genome-wide significance and none have been replicated in independent samples (Anney et al., 2012;

Anney et al., 2010; Hussman et al., 2011; Ma et al., 2009; Salyakina et al., 2010; Wang et al., 2009; Weiss, Arking, Daly, & Chakravarti, 2009). A potential reason for this is lack of sufficient statistical power due to small effect sizes expected from individual SNPs (Anney et al., 2012). Thus, larger mega-analyses are currently underway to increase statistical power through larger sample size ("Psychiatric Genomics Consortium,"). Nonetheless, combining these GWA results with other genomic data has aided in identification potential candidates pathways and networks for ASD susceptibility; Poelmans *et al.* were able to identify enrichment of three signaling networks implicating steroidogenesis, neurite outgrowth, and synaptic function, which are all functionally integrated by A-kinase anchor proteins (AKAPs) (Poelmans, Franke, Pauls, Glennon, & Buitelaar, 2013). The authors of this study suggest plausibility for AKAPs as a potential target for developing new treatments for ASD.

Rare genetic variation

Copy number variants (CNVs) are a type of genomic structural variation greater than 1kb in length, where segments of the genome are either duplicated (greater than two copies) or deleted (less than two copies). While smalls structural variation is common in the general population, larger CNVs encompassing several genes are individually rare at the locus-specific level. Genome-wide interrogation of CNVs has revealed that ASD cases have more rare CNVs than controls (Griswold et al., 2012; Pinto et al., 2010; Sanders et al., 2015). CNVs at specific regions, such as 16p11.2, have provided the most promising locus-specific genomic associations to date (Weiss et al., 2008). However, such locus-specific associations are typically not specific to ASD, often carrying risk for

additional neuropsychiatric conditions, such as ID, schizophrenia and bipolar disorder (Malhotra & Sebat, 2012). Moreover, while effect sizes are large, prevalence, even among ASD cases is low (Gaugler et al., 2014; Malhotra & Sebat, 2012). Additionally, ASD is associated with increased burden of *de novo* CNVs, rare inherited CNVs, and rare CNVs intersecting genes, particularly ASD candidate genes (Levy et al., 2011; Pinto et al., 2010; Sanders et al., 2011). Enrichment analysis of rare CNVs has implicated disruption of functional gene sets involved in cell proliferation, projection, and motility, and GTPase/Ras signaling (Pinto et al., 2010).

Single nucleotide variants (SNVs) are ultra-rare variants identified by DNA sequencing techniques. Next generation sequencing (NGS) methods have been used in more recent publications to assess this type of rare variation. Two whole-exome sequencing (WES) studies show that *de novo* SNVs, while extremely rare in the general population, are over represented among ASD probands in more than 40 genes (De Rubeis et al., 2014; Iossifov et al., 2014). Additionally, whole-genome sequencing (WGS) efforts found considerable heterogeneity in *de novo* and rare inherited mutations, even within families, with nearly 70% of affected sibling pairs harboring discordant mutations in ASD candidate genes (Yuen et al., 2015).

Epigenetic variation

Epigenetic changes are chemical modifications to the genome that control gene expression. DNA methylation, the most widely studied type of epigenetic change, is where there is an addition of a methyl group to the 5' position of cytosine. Generally, hypermethylation resulting in decreased gene expression and hypomethylation results in

increased gene expression. DNA methylation is tissue specific, may change over time, and can be modified by environmental exposures (Keil & Lein, 2016; Tordjman et al., 2014). Methylation marks are heritable and can be reversed over the lifespan (Waterland & Michels, 2007). DNA methylation and ASD have been associated, mainly in studies where DNA methylation has been isolated from brain and peripheral blood tissues. However, to date sample sizes have remained small (all studies < 100 samples) and thus additional studies are needed to understand the role of DNA methylation in ASD. Genome-wide approaches in brain tissue have implicated various genes, such as *TSAPN32*/*C11orf21*, *ZFP57*, *PRRT1*, and *SDHAP3* (Ladd-Acosta et al., 2014; Nardone et al., 2014), while candidate gene approaches have found associations with the *MeCP2, OXTR, EN-2, and SHANK3* (Gregory et al., 2009; James, Shpyleva, Melnyk, Pavliv, & Pogribny, 2013; Nagarajan, Hogart, Gwye, Martin, & LaSalle, 2006; Zhu et al., 2014). Genome-wide methylation studies using peripheral blood have also implicated various genes such as *NFYC*, *PTPRCAP*, and *MBD4* (Gregory et al., 2009; Nguyen, Rauch, Pfeifer, & Hu, 2010; Wong et al., 2014).

1.6.3. Environmental (non-genetic) risk factors

Parental age

Many studies, including meta-analyses, have linked advanced parental age to ASD in offspring (Gardener, Spiegelman, & Buka, 2009; Hultman, Sandin, Levine, Lichtenstein, & Reichenberg, 2011; Hultman, Sparen, & Cnattingius, 2002; Lampi et al., 2013; Parner et al., 2012). This has led to hypotheses about increasing risk for *de novo* mutations in gametes, epigenetic changes associated with aging, or suboptimal *in utero*

environment for the fetus through increased risk for obstetric complications and toxicant exposure (Hultman et al., 2011; Parner et al., 2012). However, since maternal and paternal ages are often highly correlated, it remains unclear whether it is advanced maternal and paternal contributes to ASD risk independently or jointly. A large population cohort study from five countries with over 30,000 ASD cases revealed that advancing maternal and paternal age were both independently associated with ASD risk (Sandin et al., 2016). However, they also found that low maternal age (< 20 years) was also associated with ASD in the child, and that joint effects were present for maternal/paternal pairs with increased difference in age (Sandin et al., 2016).

Prenatal, perinatal, and neonatal risk factors

Maternal infection

Animal studies have suggested that maternal immune activation during pregnancy affects neurodevelopment in the fetus (Patterson, 2011; Shi, Fatemi, Sidwell, & Patterson, 2003; Smith, Li, Garbett, Mirnics, & Patterson, 2007). As a result, maternal infection has been studied in several epidemiologic samples, with inconsistent findings (Brown, Jones, MacKewn, & Plank, 2008; Guinchat et al., 2012; Mamidala et al., 2013; Meldrum et al., 2013; Ornoy, Weinstein-Fudim, & Ergaz, 2015; Visser et al., 2013; Zerbo, Iosif, et al., 2013). Results from a large Danish population-based cohort suggest that common, mild infections and brief febrile episodes did not incur higher risk for ASD in offspring, but that influenza, prolonged febrile episodes and antibiotic use resulted in a two- to threefold risk (Atladottir, Henriksen, Schendel, & Parner, 2012). Similar findings were reported using California health record data, where any infection during pregnancy

was not associated with ASD, but severe infections and multiple infections were associated with ASD in the offspring (Zerbo, Qian, et al., 2013).

Smoking and alcohol use during pregnancy

Many population-based studies have investigated exposure to maternal smoking during pregnancy, with mixed results. Some studies reported positive associations with ASD diagnoses or traits (Hultman et al., 2002; Hvidtjorn et al., 2011; Indredavik, Brubakk, Romundstad, & Vik, 2007; Larsson, Weiss, Janson, Sundell, & Bornehag, 2009; Ronald, Happe, Dworzynski, Bolton, & Plomin, 2010; Tran et al., 2013; Visser et al., 2013), while others report null or trending toward negative associations (Bilder, Pinborough-Zimmerman, Miller, & McMahon, 2009; Burstyn, Sithole, & Zwaigenbaum, 2010; Kalkbrenner et al., 2012; B. K. Lee et al., 2012; Maimburg & Vaeth, 2006). Lyall *et al.* suggests interpreting reported associations with caution, and urge further, more rigorous investigation in this hypothesized risk for ASD (Lyall, Schmidt, & Hertz-Picciotto, 2014). Results from a large population cohort study suggest that smoking may be a confounding factor associated with ASD through other risk factors correlated with other demographic factors (B. K. Lee et al., 2012).

Far less work has been done in the epidemiologic investigation into alcohol consumption during pregnancy and ASD in offspring. Some have reported no association between alcohol and ASD (Eliasen et al., 2010; Lyall et al., 2014) while others have reported an inverse relationship between alcohol use during pregnancy and ASD risk (Visser et al., 2013). A review of existing literature on prenatal toxicant exposures concludes that evidence to date does not suggest an association between maternal smoking and alcohol use during pregnancy and ASD, but that future studies

should investigate the potential for this relationship more rigorously, with better exposure assessment (Lyall et al., 2014).

Obstetric complications

Results from studies reporting associations between preeclampsia and related conditions (hypertension, swelling, proteinuria, etc.) have been largely inconclusive. A meta-analysis of over fifty prenatal risk factors found that maternal obstetric complications, including preeclampsia, proteinuria, swelling, and hypertension, showed the strongest association with ASD (Gardener et al., 2009). In 2012, a review of prenatal risk factors suggested further investigation into preeclampsia in the role of ASD risk (Guinchat et al., 2012). In a large population cohort in Western Australia, no association was found between any maternal conditions and ASD, but there was weak association for ASD with comorbid ID (Langridge et al., 2013). However, maternal high blood pressure was associated with ASD in a large Finnish population cohort (Polo-Kantola et al., 2014). Others have found that preeclampsia and swelling are associated with increased ASD severity scores measuring both communication and restricted repetitive behaviors (Wallace, Anderson, & Dubrow, 2008). A more recent study implicates preeclampsia as a risk factor for both ASD and developmental delay more broadly (Walker et al., 2015). Mechanisms by which preeclampsia and related symptoms might affect fetal neurodevelopment include: enhanced inflammation, vascular damage, insulin resistance in the mother as well as decreased placental perfusion and blood and oxygen to the fetus.

Gestational diabetes increases risk for a mother to develop preeclampsia and related symptoms and has been reportedly associated with ASD diagnosis and related cognitive and adaptive behavior traits in offspring (Gardener et al., 2009; Krakowiak et al., 2012). However, a subsequent review of prenatal risk factors cautions against over interpretation of these findings and suggests further investigation is necessary into the potential link between gestational diabetes and ASD (Guinchat et al., 2012).

Birth outcomes, perinatal and neonatal conditions

A meta-analysis including neonatal factors found nearly twenty specific perinatal and neonatal complications, including fetal distress, birth injury/trauma, maternal hemorrhage, low birth weight, small for gestational age, and neonatal anemia, were associated with ASD (Gardener, Spiegelman, & Buka, 2011). Many studies have found that low birth weight and being small for gestational age is associated with ASD, however it does not seem to be specific in association as it is also linked to various other developmental disabilities, including ID and LD (Lampi et al., 2012) (Maimburg & Vaeth, 2006; Mamidala et al., 2013; Schieve et al., 2010; Schieve et al., 2015; Schieve et al., 2016; Tamaru et al., 2011). For example, Schieve *et al.* found similar population attributable fractions of low birth weight and preterm birth, ranging from 10-20%, for ASD, ID, and LD (Schieve et al., 2016). On the other hand, examining a full range of birth weights and developmental disabilities, others have found that birth weight was not associated with ASD, but was associated with various developmental delays and learning disability (Boulet, Schieve, & Boyle, 2011).

Many perinatal and neonatal conditions, such as hypoxia, respiratory distress, and neonatal jaundice have been linked to ASD risk (Bauer & Kriebel, 2013; Froehlich-

Santino et al., 2014; Gardener et al., 2009, 2011; Guinchat et al., 2012; Mamidala et al., 2013). However, findings for individual risk factors across studies are largely inconsistent, possibly due to differences in sample ascertainment and case definition (Gardener et al., 2009, 2011). For example, county-wide study of public records from Utah did not find any neonatal factors to be significantly associated with autism (Bilder et al., 2009). Moreover, findings from a population-based matched case-control study of autism suggest suboptimal birth conditions often associated with ASD are explained by low birth weight and birth defects among cases (Maimburg & Vaeth, 2006). Further work is needed to clarify either individual effects of perinatal risk factors, or understand potential common pathways to many of these risk factors that may be mediating the risk for ASD.

1.7. Methodological challenges to studying ASD

1.7.1. Overview of methodological challenges

This section will briefly review some of the challenges that accompany studying prevalence correlates, and etiology of ASD. First, we will discuss how low incidence and prevalence of ASD affects cross-sectional and prospective study designs. Specifically, we will detail the pros and cons of the two types of studies used in this dissertation: nationally representative surveys and enriched-risk prospective cohorts. Second, we discuss issues of heterogeneity (etiologic and phenotypic) and dimensionality (as ASD-related traits and risk factors that cross diagnostic boundaries). Specifically, we will review examples of both genetic and non-genetic risk factors that show specificity to phenotypic subtypes of ASD, as it pertains to comorbidity, or symptomatology. This will

be followed by a review of examples of genetic and non-genetic risk factors that span across diagnostic boundaries, either by broader non-clinical ASD traits, or affecting multiple neuropsychiatric conditions.

1.7.2. Low incidence and prevalence of ASD

Cross-sectional designs

Many cross-sectional study studies ascertain cases through clinics as a convenient way to obtain enough cases to make a statistically meaningful comparison to controls. Case status is often confirmed by clinical evaluation and phenotypically well-defined; that is, we it is we are sure a case is actually affected with ASD and is easy to obtain information on comorbid conditions and symptom profiles. However, clinically ascertained samples may lead to biased results, as demographics, risk factors and comorbid conditions may be differentially distributed among those currently seeking treatment and participating in clinical research compared to those with ASD not visiting the particular clinics recruiting for the study. Therefore, random, representative sampling is ideal for determining unbiased prevalence and associations. In this project, we used data from the National Comorbidity Survey Adolescent supplement (NCS-A), a nationally representative face-to-face survey of prevalence and correlates of DSM-IV mental disorders. The NCS-A data are geographically and socio-demographically representative of all US adolescents attending school at the time of ascertainment (Kessler et al., 2009). Therefore, we can make unbiased estimates of comorbid conditions and risk factors associated with ASD in US adolescents. However, when a disease is relatively rare, such as ASD, the case sample size will be drastically lower than

that of non-cases. Additionally, while the NCS-A was able to obtain in depth phenotypic information on a wide range of mental disorders, we rely on parent-reported data for ASD, LD, physical comorbidity, and early life risk factors. Here, we sacrifice ASD case sample size and in-depth phenotyping for representativeness.

Prospective designs

Prospective studies allow us to study the development of disease before symptom onset is apparent by ascertaining unaffected individuals and following them over time while collecting relevant data on risk factors and markers of disease. This is beneficial to collect time-relevant exposures and assessments. However, when your disease of interest is relatively rare, you would have to follow many subjects in order to obtain enough cases to draw meaningful conclusions from the study population. This is often cost prohibitive and inefficient when studying rare diseases. High-risk cohort designs, where participants are selected based on having higher risk of developing the disease of interested compared to the general population, offer an effective alternative to a traditional cohort designs. Because siblings of children with ASD are nearly twenty times more likely to develop ASD compared to the general population (Ozonoff et al., 2010), ASD high-risk studies often recruit baby siblings of children with ASD in order to observe development and collect behavioral and biosample data before a diagnosis can be made (Newschaffer et al., 2012). In this project, we use two high-risk cohorts, the Early Autism Risk Longitudinal Investigation (EARLI) and the Infant Brain Imaging Study (IBIS) that collected measures of cognitive and motor development longitudinally from age 6 to 36 months. Here, we sacrifice representativeness and sample size for rich phenotypic data and enrichment for observing developmental traits associated with ASD.

1.7.3. Heterogeneity and dimensionality

Within-ASD heterogeneity

ASD is a highly heterogeneous condition, with respect to symptom profile, severity, and comorbid conditions. Several genetic and non-genetic risk factors for ASD have shown a differential effect dependent upon ASD subgroups. For example, maternal and paternal age was differentially associated with ASD subtypes, where advanced paternal age was associated with more severe autistic disorder, while advanced maternal age was associated with less severe subtypes of ASD (Lampi et al., 2013). An examination of rare genic CNVs among ASD cases found that different sets of CNVs affected different sub-phenotypes, where CNVs in genes previously implicated in ASD and ID were associated with communication and language abilities, while CNVs in differentially brain expressed regions were more closely associated with adaptive functioning (Merikangas et al., 2015). Such findings highlight the importance of considering phenotypic heterogeneity in etiologic research of ASD.

Non-specificity across ASD diagnostic boundaries

As discussed previously, many risk factors (genetic and non-genetic) and comorbidities associated with ASD are also associated with other neurodevelopmental disorders, suggesting there may be underlying common traits or pathways to disease crossing diagnostic boundaries (Schieve et al., 2012). For example, a study comparing birth outcomes between ASD and ID found that preterm birth, low birth weight, small for gestational age, and low Apgar scores were associated with both ASD and ID

independently (Schieve et al., 2015). Additionally, several genetic risk factors are non-specifically associated with ASD. Rare CNVs associated with ASD often account for only a fraction of individual ASD risk, but are also associated other neuropsychiatric traits such as schizophrenia and ID (Malhotra & Sebat, 2012). Additional studies have found that ASD-implicated genetic variants are also associated with cognitive and subclinical ASD-related traits in the general population (Mannik et al., 2015; Robinson et al., 2016).

1.8. Specific Aims

This research aimed to examine comorbidity, environmental, and genetic risk factors associated with ASD in the context of general neurodevelopment. The specific aims were:

1. To evaluate lifetime presence of physical and mental health conditions associated with parent reported ASD and LD in the National Comorbidity Survey Adolescent Supplement (NCS-A), a population-based, nationally representative sample of US adolescents (Chapter 2).
2. To estimate prevalence and association of prenatal, perinatal, and neonatal risk factors with ASD and LD in a nationally representative, population-based sample of US adolescents (NCS-A) (Chapter 3).
3. To identify common ASD-related genetic variants associated with differential trajectories of cognitive and motor development, as measured by the Mullen Scales of Early Learning (MSEL), among infant high-risk ASD siblings from the Early Autism Risk Longitudinal Investigation (EARLI) and Infant Brain Imaging Study (IBIS) enriched risk cohorts (Chapter 4).

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CHAPTER 2:

PHYSICAL AND MENTAL COMORBIDITY OF AUTISM SPECTRUM DISORDER AND LEARNING DISORDER IN U.S. ADOLESCENTS

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ABSTRACT

Objective: Autism spectrum disorder (ASD) and learning disorder (LD) are highly comorbid neurodevelopmental disorders (NDDs). ASD is associated with an array of physical and mental conditions in clinically ascertained samples. We report prevalence and odds of physical and mental conditions associated with ASD and LD in a nationally representative population-based sample of US adolescents.

Method: Mutually exclusive groups of adolescents with parent survey data from the National Comorbidity Survey - Adolescents Supplement (NCS-A) (n=6,296) with ASD with or without LD and LD without ASD were compared to those without either ASD or LD. Estimates were weighted to represent the population of US adolescents at the time of enrollment.

Results: Allergies (aOR = 3.48, p = 0.004), acne (aOR = 3.25, p = 0.02), heart problems (aOR = 4.08, p = 0.02), and early morning awakening (aOR = 2.44, p = 0.04) were specifically associated with ASD. Gastrointestinal problems (ASD: aOR = 5.60, p = 0.004; LD: aOR = 1.70, p = 0.03), epilepsy/seizures (ASD: aOR = 9.97, p < 0.001; LD: aOR = 2.82, p = 0.001), and ADHD (ASD: aOR = 8.38, p < 0.001; LD: aOR = 6.13, p < 0.001) were associated with both ASD and LD.

Conclusion: Several conditions are specifically associated with ASD. However, gastrointestinal problems, epilepsy/seizures, and ADHD are associated with both ASD and LD in our population-based sample, indicating an association with general neurodevelopment rather than ASD specifically. Findings have important implications for clinical evaluation and provide potential etiologic insight into NDDs.

INTRODUCTION

Autism spectrum disorder (ASD) is an early-onset neurodevelopmental disorder (NDD) characterized by social and communication deficits and restrictive and repetitive behaviors or interests (American Psychiatric Association, 2013). ASD affects approximately 1.5% of children in the US, many of whom also suffer from learning disorder (LD) (CDC, 2014).

Previous reports of clinically ascertained samples of youth with ASD have suggested higher than expected rates of comorbid physical conditions affecting a variety bodily systems, including gastrointestinal, autoimmune/inflammatory disorders, sleep disturbances and neurological disorders (Bauman, 2010; Matson & Goldin, 2013). Similarly elevated rates of comorbidity with a range of physical conditions and healthcare service use have been observed in community-based samples of youth with ASD (Gurney, McPheeters, & Davis, 2006; Schieve et al., 2012). ASD has also been associated with a variety of mental disorders such as attention deficit/hyperactivity disorder (ADHD) (Matson, Rieske, & Williams, 2013; Memari, Ziaee, Mirfazeli, & Kordi, 2012), anxiety, and mood disorders (Joshi, Biederman, et al., 2013; Joshi, Wozniak, et al., 2013; McPheeters, Davis, Navarre, & Scott, 2011) in clinical and community-based samples. Several of these conditions have also been reported at increased prevalence for several other neurodevelopmental disorders, such as ADHD and intellectual disability (ID) (Schieve et al., 2012).

Previous work suggests mental and physical conditions may cluster differentially among youth with ASD resulting in comorbidity-specific trajectories that may reflect distinct ASD subtype etiologies.(Doshi-Velez, Ge, & Kohane, 2014) Hypotheses have

been made about the link between ASD and comorbid conditions when studying these conditions individually in clinical samples; however, understanding the full range of physical conditions associated with ASD in a non-biased population sample could improve hypothesis generation regarding underlying pathways to neurodevelopmental disorder. Accurate profiles of comorbid risk can also potentially improve implementation of clinical care.

Most previous research concerning ASD physical and mental comorbidity has been conducted in clinically referred samples, which may lead to elevated estimates of comorbidity due to differential treatment-seeking among those with comorbid conditions. Population-based studies to date have not included direct evaluation of the full range of common mental disorders in ASD youth. Furthermore, there is evidence for increased rates of many conditions shared across several neurodevelopmental disorders rather than comprising manifestations specifically associated with ASD.(Schieve et al., 2012) Specificity of comorbidity of mental and physical conditions with ASD versus broader manifestations of neurodevelopmental disorders, such as LD, has not been established.

In the current study, we estimate the prevalence and adjusted odds of parent-reported physical conditions and directly evaluate mental disorders among mutually exclusive groups of youth with ASD (with or without LD) and LD (without ASD) in a nationally representative, population-based sample of adolescents. Additionally, we examine the clinical impact and patterns of service use among these neurodevelopmental groups.

METHODS

Sample

The National Comorbidity Survey Adolescent Supplement (NCS-A) is a nationally representative household survey of adolescent mental health in the Unites States. Recruitment methods, sampling and weighting strategies have been described in detail elsewhere.(Kessler et al., 2009) Adolescents aged 13-17 were recruited via either household or school-based sampling schemes. Professional interviewers administered in-person, computer-assisted personal interviews in adolescents' homes between February 2001 and January 2004. One parent/guardian was asked to complete a self-administered questionnaire, the Parent Self-Administered Questionnaire (PSAQ), consisting of questions pertaining to the adolescent's medical and developmental history as well as parent and household characteristics. Informed assent and consent was obtained from each participating adolescent and his/her parent or legal guardian. The Harvard Medical School and University of Michigan Human Subjects Committees approved all study procedures.

Measures

Neurodevelopmental Disorders

Presence of ASD and LD were evaluated by parent response to a single yes/no question on in the PSAQ for each condition: "Has [the adolescent] ever had […] developmental disorders (such as autism, Asperger's, or pervasive developmental disorder)?" and "Has [the adolescent] ever had […] learning disability?" A positive response to either question prompted the parent to report age of onset, whether the

adolescent has ever received treatment for this problem by a doctor, and whether the adolescent has experienced this problem in the past 12 months.

Comorbid Physical Conditions

Lifetime presence of chronic physical conditions, including allergies, asthma, severe stomach problems, seizures/epilepsy, headache/migraine, acne, skin conditions (such as eczema or psoriasis), and heart problems were assessed by parent response to a series of yes/no questions about the adolescent's health. Enuresis was coded as present if the parent reported that the child had ceased bed wetting after age 5. Sleep problems were assessed by adolescent response to a series of three questions in the chronic conditions module of the CIDI pertaining to difficulty initiating and maintaining sleep as well as frequent early-morning awakening.

Comorbid Mental Disorders

Comorbid mental disorders were assessed by direct household interviews with adolescents on the World Health Organization Composite International Diagnostic Interview (CIDI) 3.0 (Robins et al., 1988), a structured interview for the assessment of ICD-10 and DSM-IV criteria for mental disorders. DSM-IV criteria were applied for comprehensive domains of mental disorders including major depressive disorder, bipolar I, bipolar II, generalized anxiety disorder, social phobia, separation anxiety disorder, panic disorder, post-traumatic stress disorder, anorexia, bulimia, alcohol abuse/dependence, drug abuse/dependence, conduct disorder, intermittent explosive disorder, and attention deficit hyperactivity disorder.(Merikangas et al., 2010) Diagnoses were then grouped by disorder class (mood, anxiety, eating, substance, conduct/behavioral, and ADHD) for analysis as dependent variables.

Medication and Services Use

Adolescents were asked whether they had ever received disorder-specific treatment immediately after answering diagnostic questions for each DSM-IV disorder category as part of the CIDI. In a separate interview module focusing on services, all respondents were asked whether they ever received service for emotional or behavioral problems and the settings in which they had received those services. Parents were asked similar questions pertaining to their child's healthcare/social services use to those administered to the adolescent. Based on endorsement by either adolescent or parent, service use was classified into 5 categories: 1) mental health specialty, 2) general medical, 3) complementary and alternative medicine, 4) juvenile justice, and 5) school-based services.

Adolescents were shown a comprehensive list of over 300 brand and generic names of pharmacologic medications and were asked to indicate which ones they took on a regular basis in the past 12 months. Responses were recorded and grouped into 6 categories: anti-depressants, anti-psychotics, anxiolytics, mood stabilizer/anticonvulsants, stimulant, and miscellaneous.

Impairment

Overall functional impairment was measured by total difficulties score of the Strengths and Difficulties Questionnaire (SDQ), which evaluates emotional, attentional, peer relationship, and conduct problems in a dimensional manner suitable for population-based studies.(Goodman, 1997)

Statistical Methods

Statistical analyses were carried out in R version 3.1.1 (Lumley, 2014; R Core Team, 2014). Survey-weighed multiple logistic regression was used to calculate associations between parent-reported NDD status - ASD with or without LD (ASD) and LD without ASD (LD-only) - and physical condition or mental disorder. All analyses adjusted for age, sex, race/ethnicity, and household income of the adolescent and were weighted to represent the national population of adolescents attending school at the time of study recruitment. Methods to develop weights are described in detail elsewhere (Kessler et al., 2009). Missing data were assumed to be missing completely at random and were handled by model-wise deletion.

RESULTS

The weighted prevalence of parent-reported ASD and LD among adolescents with parent report data in the NCS-A was 0.7% (SE: 0.1%) and 14.3% (SE: 0.7%), respectively. The prevalence of LD among ASD cases was 70.0% (SE: 8.0%) and 13.7% (SE: 0.7%) among non-ASD cases. The prevalence of ASD among LD cases was 3.6% (SE: 0.9%), and 0.3% (SE: 0.1%) among non-LD cases. By statistical comparison group, 0.7% (SE: 0.1%) had ASD with or without LD while and 13.7% (SE: 0.7%) had LD without ASD; 85.6% (SE: 0.7%) had neither ASD nor LD.

Table 2.1 shows the comparison of demographic characteristics of the weighted sample by ASD and LD status, with statistical comparisons to the unaffected (non-NDD) group. Both NDD-affected groups were predominantly male compared to the non-NDD group: ASD: 85.5% v. 48.2%, $X^2_1 = 15.50$, $p < 0.001$; LD-only: 66.7% v. 48.2%, $X^2_1 =$

29.45, $p < 0.001$. The ASD group also differed significantly from the non-NDD group on distribution of race/ethnicity (X^2_3=7.32, $p < 0.001$). The LD-only group differed significantly from the non-NDD group by household income (X^2_3=6.78, p=0.001) and parent marital status (X^2_3=3.79, p=0.02). The NDD subgroups did not differ from the non-NDD group in mean adolescent age, parent educational status, English spoken in the home, geographic region, or urban environment. Additional comparisons of number of siblings and birth order revealed no differences between any NDD group and the non-NDD group. Gender, age, household income, and parent education were included as covariates in subsequent analyses.

Table 2.2 presents weighted estimates of prevalence and demographic-adjusted odds ratios of comorbid physical conditions among NDD subgroups. The ASD group was more likely to endorse allergies/hay fever (aOR=3.43, 95% CI=1.57-7.48, p=0.004), acne (aOR=3.25, 95% CI=1.30-8.13, p=0.02) and heart problems (aOR=4.08, 95% CI=1.27-13.13, p=0.02) compared to the non-NDD group, while the LD-only group did not differ from the non-NDD group on these conditions. ASD and LD-only NDD groups had higher risk for both severe stomach problems (ASD: aOR= 5.60, 95% CI = 1.86-16.85, p= 0.004; LD-only: aOR= 1.70, 95% CI = 1.09-2.66, p=0.03) and epilepsy/seizures (aOR=9.97, 95% CI=3.24-30.69, p<0.001); LD-only: aOR= 2.82, 95% CI = 1.58-5.05, p= 0.001). The LD-only subgroup showed increased odds for headache/migraine (aOR = 1.41, 95% CI = 1.04-1.91, p=0.03), enuresis (aOR = 1.72, 95% CI = 1.14-2.62, p=0.02), while ASD group was not significantly associated with any of these conditions. There were no differences in risk of asthma or skin conditions other than acne among either NDD-affected group compared to non-NDDs. The ASD group

had higher odds of experiencing frequent early morning awakening (aOR=2.44, 95% CI=1.07-5.56, p=0.04) in the past 12 months, while LD-only adolescents had higher odds of any sleep problem (aOR = 1.49, 95% CI= 1.17-1.89, p=0.003), and specifically, sleep initiation (aOR = 1.47, 95% CI = 1.17-1.89, p=0.003).

Table 2.3 presents weighted estimates of prevalence and demographic-adjusted odds ratios of comorbid mental disorders by NDD subgroup. Both the ASD (aOR = 8.38, 95% CI = 4.15-16.90, p<0.001) and LD (aOR = 6.13, 95% CI = 4.47-8.41, p<0.001) affected groups had over six-fold increased odds of meeting ADHD diagnostic criteria. The LD-only group showed increased odds of comorbid anxiety disorder (aOR = 1.28, 95% CI = 1.03-1.59, p=0.03) and conduct/behavioral disorder (aOR = 1.87, 95% CI = 1.40-2.50, p<0.001). Neither NDD-affected group had significantly higher odds of comorbid mood, eating, or substance abuse/dependence disorders compared with the non-NDD group.

Table 2.4 presents weighed estimates of prevalence and demographic-adjusted odds of past 12-month medication use and lifetime health and social service use by NDD subgroup. The ASD group had nearly 8.5 times higher odds (aOR = 8.49, 95% CI = 3.43-21.01, p<0.001) of medication use in the past 12 months while the LD-only group had over 3.5 times higher odds (aOR = 3.55, 95% CI = 2.38-5.30, p<0.001) compared to the non-NDD group. Medication subcategory analyses (Table 2.4) revealed that ASD status was associated with every subcategory of 12-month medication use and LD status was associated with nearly every subcategory (excluding anti-psychotic and anxiolytic). Regarding services, the ASD group was more likely to receive mental health specialty, general medical, and school services compared to non-NDD adolescents. The LD-only

group was also more likely to have received these services as well as complementary and alternative medicine (CAM) and juvenile justice services. Both the ASD (aOR = 5.93, 95% CI = 2.65-13.23, $p<0.001$) and LD (aOR = 4.16, 95% CI = 3.10-5.59, $p<0.001$) group were significantly more likely to score in the 90th percentile on the difficulties composite of the SDQ.

DISCUSSION

These findings provide estimates of ASD physical and mental comorbidity and healthcare service use in a nationally representative sample of US adolescents. Overall prevalence rates of ASD (0.7%) and LD (14.3%) were similar to those found in other population-based studies collected during the same time period (CDC, 2007), with the majority of ASD adolescents (70%) experiencing concurrent LD (Gurney et al., 2006; Schieve et al., 2012). ASD and LD groups were characterized by different patterns of comorbidity, which may have important implications for etiologic inferences and risk factor identification.

The high prevalence of LD among ASD cases calls for identification of comorbid conditions specific to ASD. Increased risk for gastrointestinal problems and epilepsy/seizures, two physical conditions suspected to be etiologically related to ASD,(Amiet et al., 2008; Bauman, 2010; Mayer, Padua, & Tillisch, 2014) were present in both the ASD and LD groups. This suggests a potential overarching neurodevelopmental component to these conditions that is not specific to ASD alone. Furthermore, LD in the absence of ASD was uniquely associated with several physical and mental conditions, indicating a potential need for increased awareness among parents and healthcare

providers of specific mental and physical health problems among adolescents with LD. Accurate understanding of NDD comorbidity can potentially improve implementation of clinical care as well as inform genetic and other etiologic research. Further prospective research is needed to establish temporality and identify potential biological mechanisms resulting in cross-domain comorbidity in neurodevelopmental disorders.

ASD cases were nearly six times more likely and LD-only nearly twice as likely to have severe GI problems compared to their non-NDD counterparts. Many previous studies have indicated increased rates of GI problems among ASD children, with a recent meta-analysis providing consensus, (McElhanon, McCracken, Karpen, & Sharp, 2014) but the link is not well understood.(Coury et al., 2012) Furthermore, little is known about the prevalence of GI problems specifically among youth with LD alone. There is growing evidence suggesting a link between alterations in the gut microbiome and neurodevelopment leading to social, behavioral, and cognitive impairment in ASD (Coury et al., 2012; Mayer et al., 2014). These findings suggest that this link may extend to other NDDs as well.

We also found a similar pattern of association between both NDD subgroups and risk for epilepsy and seizures. Previous reports have shown increased prevalence rates of seizures in ASD with a range from 5-40%.(Bauman, 2010) A meta-analysis of 23 studies suggested that risk is affected by age, gender and cognitive ability, with greater rates of epilepsy in ASD girls with intellectual disability (ID)(Amiet et al., 2008). While we do not have a measure of ID in the current study, it is interesting to note that we find the highest prevalence of epilepsy/seizures in ASD (18.0%) and elevated to a lesser extent, yet still significantly, in LD alone (4.7%). While it remains unclear whether the

associations between these NDDs and both GI problems and seizures are causal or comorbid, the association has been consistently found in previous studies, particularly in the only prior population based sample of Schieve *et al* (Schieve et al., 2012), who also reported increased risks for seizures and gastrointestinal problems across all developmental disabilities examined in the National Health Interview Survey, including autism, autism with ID, and LD as well as ADHD. More recently, these associations also emerged from the application of an unsupervised clustering statistical approach to investigate comorbidity in ASD based on electronic medical records.(Doshi-Velez et al., 2014) Therefore, these disorders may reflect a broader manifestation of the multi-system impact of neurodevelopmental problems or their risk factors.

ADHD, another NDD, was associated with both ASD and LD. These findings support those of many studies have reported increased ADHD symptoms and diagnoses in ASD (Matson et al., 2013; Memari et al., 2012; Schieve et al., 2012) and LD (Pham & Riviere, 2015) children. Further, this association confirms previously reported evidence for shared genetic underpinnings across these and other neurodevelopmental disorders (Pettersson, Anckarsäter, Gillberg, & Lichtenstein, 2013; Posthuma & Polderman, 2013).

Several conditions, including early morning awakening, allergy, acne, and heart problems, were specifically associated with ASD but not LD. Early morning awakening sleep problems (Giannotti, Cortesi, Cerquiglini, Vagnoni, & Valente, 2011) and allergy (de Theije et al., 2014; Theoharides, 2013) have both been previously shown to be associated with ASD (Bauman, 2010; Gurney et al., 2006; Schieve et al., 2012). Sleep disorders have also been linked to ADHD (Tsai & Huang, 2010) and we find increased prevalence of any sleep problem in our LD sample; therefore, these findings suggest that

sleep disruption may be a general problem among youth with NDDs. Amelioration of sleep problems could potentially lead to improvements in cognitive and adaptive functioning for multiple neurodevelopmental disorders (Taylor, Schreck, & Mulick, 2012).

While allergies have previously been associated with neurodevelopment in general rather than with ASD specifically, (de Theije et al., 2014; Julvez et al., 2009; Meldrum et al., 2012) our results do not indicate a common comorbidity spanning ASD and LD. It is possible that the link between allergy and neurodevelopment does not encompass pathways associated with the specific deficits of LD, but perhaps more severe cognitive or social/communication deficits. Adolescents with ASD were also nearly three times more likely to have acne. It has recently been posited that imbalances in the human microbiome similar to those associated with ASD and GI problem may also lead to acne and various other skin conditions.(Muszer, Noszczynska, Kasperkiewicz, & Skurnik, 2015)

Heart problems were reported in nearly 13% of ASD cases. Previous research has demonstrated autonomic hyper arousal, with elevated basal heart rate and atypical reactivity to social tasks among those with ASD.(Kushki, Brian, Dupuis, & Anagnostou, 2014) Furthermore, some genetic deletion syndromes that frequently present with autistic features also present with cardiac defects.(Celestino-Soper et al., 2012; Niklasson, Rasmussen, Óskarsdóttir, & Gillberg, 2002) However, we could not examine this further because of the lack of information on genetic syndromes in this sample and no subtypes of cardiac problems were reported by the parent.

By contrast, LD-affected children without comorbid ASD were significantly more likely to have comorbid enuresis, sleep problems (primarily initiation), severe headaches/migraine, and several mental disorders including anxiety disorders, conduct and behavioral disorders compared to non-NDD adolescents. These conditions may be a common manifestation of delayed neurodevelopmental maturation that could potentially result from common risk factors or consequences of LD. In either case, LD-only adolescents clearly carry an increased burden of chronic conditions that is distinct from that of adolescents suffering from ASD. Healthcare providers and educators should be aware that LD adolescents are at higher risk for these health problems, which could influence implementation and effectiveness of healthcare and school-based interventions.

It is possible that differences in sample size/power between NDD subgroups could explain why we see some associations in the larger LD-only group, but not the ASD subgroups. However, extending the significance threshold to $p < 0.10$ in the smaller ASD subgroup did not show significant associations, indicating uniqueness for the LD-only group. This may suggest that the LD reported in ASD children may be biologically distinct from the LD reported in children without ASD, and that this subgroup is at risk for a different, yet still substantial burden of chronic health conditions. These findings have particular relevance to the awareness of, and services for adolescents with LD. Whereas ASD services are primarily funded through Medicaid or other state based funding for youth, either specifically for ASD or through Serious Emotional Disorders mandates, resources for the recognition and treatment of LD are primarily in the education system. In light of the high magnitude of comorbidity between these

conditions, such differential service streams for youth are likely to induce uncoordinated and fragmented care for such youth.

NDD status and presence of physical conditions were based solely on parent report rather than direct parental interview. This may have led to some misclassification compared to direct evaluation because of our inability to probe responses. However, previous studies support the validity of parent report of such conditions (Centers for Disease Control and Prevention, 2006; Daniels et al., 2012). In addition, statistical comparison of ASD and LD comorbidity risk profiles was impeded by the vastly different sample sizes between affected groups. Therefore, we considered overall patterns in point estimates and directionality, rather than relying solely upon p-values and statistical significance. These disorders are based on DSM-IV diagnostic criteria that do not reflect changes implemented in the DSM-5. However, because the new criteria were designed to measure the same psychological phenomena in a dimensional manner, we expect that our conclusions are still likely to be relevant (Huerta, Bishop, Duncan, Hus, & Lord, 2012).

These findings are based on a nationally representative sample of adolescents ascertained without respect to NDD or comorbidity status, thus limiting potential for inflated comorbidity prevalence estimates. Of note, these are the first prevalence estimates of directly assessed mental disorders among ASD and LD in a nationally representative sample of U.S. adolescents. While the CIDI has not been validated specifically in ASD populations, all ASD participants in the current study had sufficient functional status to attend school and to complete the interview process. Second, information collected on both physical and mental conditions simultaneously in the NCS-

A provided a rare opportunity for extensive examination of physical-mental comorbidity. The extensive information collected from parent questionnaires also permitted evaluation of specificity through our inclusion of LD, another NDD highly comorbid with ASD, as a comparison group.

This study utilized nationally representative population-based sample to provide estimates of physical and directly assessed mental disorders among adolescents with NDDs in the US. While some conditions appear to be specifically associated with ASD, others may reflect broader manifestations of NDDs. Future research should examine these patterns of comorbidity as potential clues to shared etiology. Furthermore, these findings highlight the importance of clinical characterization of the full spectrum of both physical and mental disorders that may impact functioning among those with NDDs in adolescence and, potentially, throughout the life course.

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Table 2.1. Demographic characteristics of NCS-A adolescents by ASD and LD status [a]

Demographic Characteristics	Autism Spectrum Disorder (N = 51)		Learning Disorder Only (N = 879)		None (N = 5,366)
	% (SE)	X^2, p [b]	% (SE)	X^2, p [b]	% (SE)
Gender		15.50, <0.001		29.45, <0.001	
male	85.5 (5.0)		66.7 (3.1)		48.2 (1.3)
female	14.5 (5.0)		33.3 (3.1)		51.8 (1.3)
Age (mean)		-0.34[c], 0.74		1.01[c], 0.32	
	15.1 (0.33)		15.3 (0.12)		15.2 (0.07)
Race/Ethnicity		7.32, <0.001		0.41, 0.75	
Hispanic	3.9 (2.0)		12.9 (1.9)		14.7 (1.5)
Black	26.6 (7.4)		16.0 (2.0)		14.4 (1.1)
Other	14.4 (2.7)		6.5 (2.7)		4.7 (0.7)
White	55.1 (8.1)		64.5 (4.2)		66.1 (1.9)
Household Income [d]		1.45, 0.24		6.78, 0.001	
PIR ≤ 1.5	30.2 (9.9)		19.6 (2.5)		13.2 (1.1)
1.5 < PIR ≤ 3	22.0 (8.9)		20.2 (2.7)		19.2 (0.8)
3 < PIR ≤ 6	29.7 (8.6)		24.7 (2.5)		34.1 (1.2)
PIR > 6	18.1 (6.4)		35.6 (3.0)		33.6 (1.6)
Parent Marital Status		1.23, 0.31		3.79, 0.02	
Married/ cohabiting	45.6 (10.1)		62.3 (2.3)		69.7 (1.4)
Previously married	19.4 (8.5)		19.3 (2.1)		15.7 (0.7)
Never married	8.1 (4.6)		3.9 (1.0)		4.4 (0.6)
Unknown	26.8 (9.7)		14.6 (1.9)		10.3 (0.9)
Parent Education		1.82, 0.16		1.73, 0.18	
Less than high school	29.0 (10.4)		16.6 (2.2)		11.2 (0.9)
High school graduate	20.2 (8.1)		30.6 (2.8)		29.0 (1.2)
Some college	11.0 (4.3)		20.7 (2.1)		21.7 (1.0)
College graduate	39.8 (9.8)		32.2 (3.8)		38.1 (1.6)
English spoken at home		0.22, 0.64		3.81, 0.06	
	85.5 (8.9)		86.1 (2.2)		81.3 (0.9)
Geographic region		0.37, 0.78		1.61, 0.20	
Northeast	25.9 (8.4)		17.0 (2.2)		18.4 (1.8)
Midwest	23.9 (8.5)		21.5 (2.4)		23.5 (2.1)
South	30.9 (7.5)		36.1 (3.9)		36.1 (2.6)
West	19.2 (9.0)		25.4 (2.4)		22.0 (2.0)
Urban environment		1.95, 0.15		2.03, 0.14	
Metropolitan	32.0 (8.9)		41.1 (3.7)		46.7 (2.4)
Rural	32.7 (9.4)		44.9 (3.9)		38.7 (3.2)
Other	35.4 (9.8)		14.0 (2.8)		14.5 (2.3)

[a] Percentages are weighted to represent the general population of US adolescents
[b] Test statistic and p-value reflect comparison to ASD- and LD-unaffected group
[c] t-value
[d] Household income as measured by the income-to-poverty ratio (PIR)

Table 2.2. Prevalence and adjusted odds ratios of comorbid physical conditions [a]

Physical Condition	Autism Spectrum Disorder (N = 51)	Learning Disorder Only (N = 879)	None (N = 5,366)	Autism Spectrum Disorder		Learning Disorder Only	
	% (SE)	% (SE)	% (SE)	aOR[b] (95% CI)	*p*	aOR[b] (95% CI)	*p*
Allergy	58.98 (9.5)	32.8 (3.3)	31.1 (1.1)	3.43 (1.57, 7.48)	0.004	1.09 (0.78, 1.51)	0.63
Asthma	23.84 (8.0)	16.7 (2.5)	16.9 (0.8)	1.51 (0.62, 3.66)	0.37	0.97 (0.68, 1.39)	0.88
Severe stomach problems	16.50 (6.8)	6.1 (1.2)	3.7 (0.4)	5.60 (1.86, 16.85)	0.004	1.70 (1.09, 2.66)	0.03
Epilepsy or Seizures	18.02 (7.8)	4.7 (1.2)	1.7 (0.3)	9.97 (3.24, 30.69)	<0.001	2.82 (1.58, 5.05)	0.001
Frequent headaches or migraines	24.42 (8.0)	20.6 (2.5)	15.7 (0.6)	1.76 (0.75, 4.12)	0.20	1.41 (1.04, 1.91)	0.03
Acne	24.05 (8.2)	11.4 (1.4)	8.8 (0.5)	3.25 (1.30, 8.13)	0.02	1.25 (0.87, 1.78)	0.24
Skin conditions (such as eczema, psoriasis)	16.41 (7.4)	8.1 (1.5)	8.1 (0.6)	2.59 (0.84, 8.00)	0.11	1.06 (0.69, 1.64)	0.79
Heart problems	12.95 (6.3)	4.2 (1.1)	2.7 (0.4)	4.08 (1.27, 13.13)	0.02	1.41 (0.79, 2.52)	0.25
Enuresis	17.25 (7.3)	17.6 (2.6)	10.0 (0.5)	1.44 (0.50, 4.11)	0.50	1.72 (1.14, 2.62)	0.02
Sleep problems (any)	37.89 (8.9)	40.3 (2.3)	32.5 (1.5)	1.48 (0.66, 3.31)	0.34	1.49 (1.17, 1.89)	0.003
Initiation	20.50 (7.1)	29.5 (2.0)	23.6 (1.2)	1.01 (0.44, 2.33)	0.97	1.47 (1.15, 1.87)	0.003
Maintenance	11.79 (7.8)	15.2 (2.2)	13.9 (1.1)	1.02 (0.23, 4.60)	0.98	1.21 (0.79, 1.84)	0.39
Early morning awakening	31.08 (8.4)	21.1 (2.4)	16.4 (1.1)	2.44 (1.07, 5.56)	0.04	1.38 (0.96, 1.98)	0.09

[a] All estimates are weighted to represent the general population of US adolescents
[b] aOR = odds ratios calculated from weighed logistic regression models adjusted for gender, age, race/ethnicity and household income, compared to the ASD- and LD-unaffected group.

Table 2.3. Prevalence and adjusted odds ratios of comorbid mental disorders [a]

Mental Disorder Category	Autism Spectrum Disorder (N = 51)	Learning Disorder Only (N = 879)	None (N = 5,366)	Autism Spectrum Disorder		Learning Disorder Only	
	% (se)	% (se)	% (se)	aOR [b] (95% CI)	*P*	aOR [b] (95% CI)	*p*
Mood disorders	3.19 (2.0)	15.2 (1.6)	13.8 (1.1)	0.29 (0.08, 1.05)	0.07	1.26 (0.95, 1.68)	0.11
Anxiety disorders	23.63 (6.7)	28.2 (2.2)	25.2 (0.9)	1.10 (0.51, 2.36)	0.81	1.28 (1.03, 1.59)	0.03
Eating disorders	0.91 (0.6)	4.0 (0.9)	3.0 (0.5)	0.48 (0.12, 1.85)	0.29	1.72 (0.95, 3.10)	0.08
Substance abuse/dependence	9.56 (4.1)	12.3 (1.9)	11.2 (0.9)	0.81 (0.31, 2.14)	0.68	0.99 (0.69, 1.43)	0.95
Conduct/Behavioral	21.98 (7.5)	28.8 (3.0)	17.2 (0.8)	1.32 (0.59, 2.93)	0.51	1.87 (1.40, 2.50)	<0.001
ADHD	41.63 (9.0)	30.5 (3.1)	5.9 (0.4)	8.38 (4.15, 16.90)	<0.001	6.13 (4.47, 8.41)	<0.001

[a] All estimates are weighted to represent the general population of US adolescents
[b] aOR = odds ratios calculated from weighed logistic regression models adjusted for gender, age, race/ethnicity and household income, compared to the ASD- and LD-unaffected group.

Table 2.4. Prevalence and association of 12-month medication and lifetime services use [a]

Healthcare and social services	Autism Spectrum Disorder Only (N = 14)	Learning Disorder Only (N = 879)	None (N = 5,366)	Autism Spectrum Disorder		Learning Disorder Only	
	% (se)	% (se)	% (se)	aOR [b] (95% CI)	*P*	aOR [b] (95% CI)	*p*
Medications							
Any Medication	29.55 (8.2)	15.9 (2.1)	5.2 (0.5)	8.49 (3.43, 21.01)	<0.001	3.55 (2.38, 5.30)	<0.001
Anti-depressant	11.85 (6.0)	6.1 (1.5)	3.1 (0.4)	5.40 (1.44, 20.21)	0.02	2.24 (1.17, 4.29)	0.02
Anti-psychotic	4.65 (2.6)	1.1 (0.6)	0.3 (0.1)	14.15 (2.64, 75.88)	0.004	3.29 (1.03, 10.55)	0.05
Anxiolytic	2.41 (1.7)	0.5 (0.3)	0.5 (0.1)	9.48 (1.91, 46.92)	0.009	1.13 (0.3, 4.27)	0.86
Mood Stabilizer/Anticonvulsant	7.25 (4.3)	0.8 (0.4)	0.1 (0.0)	51.75 (11.42, 234.53)	<0.001	4.75 (1.39, 16.22)	0.02
Stimulant	15.81 (7.6)	10.1 (1.9)	1.8 (0.3)	8.05 (2.25, 28.90)	0.003	5.63 (3.2, 9.91)	<0.001
Miscellaneous medications	13.41 (6.1)	1.3 (0.5)	0.3 (0.1)	42.19 (9.66, 184.33)	<0.001	3.27 (1.35, 7.96)	0.01
Services							
Mental Health Specialty	51.71 (9.5)	47.5 (3.1)	27.0 (1.1)	3.26 (1.44, 7.38)	0.007	2.50 (2.00, 3.13)	<0.001
General Medical	24.67 (6.7)	27.0 (2.5)	10.4 (0.7)	2.87 (1.30, 6.34)	0.01	3.17 (2.24, 4.49)	<0.001
CAM [c]	11.41 (4.5)	12.4 (2.1)	6.5 (0.6)	2.43 (1.01, 5.85)	0.06	2.20 (2.24, 4.49)	0.001
Juvenile Justice	14.09 (7.0)	10.0 (2.0)	4.2 (0.6)	3.28 (0.96, 11.19)	0.07	2.19 (1.43, 3.34)	0.001
School Service	75.84 (4.9)	48.8 (2.3)	17.3 (0.9)	14.35 (3.19, 8.45)	<0.001	4.35 (3.61, 5.25)	<0.001
SDQ Difficulty Composite	38.11 (9.4)	28.04 (3.0)	8.44 (0.8)	5.93 (2.65, 13.23)	<0.001	4.16 (3.10, 5.59)	<0.001

[a] All estimates are weighted to represent the general population of US adolescents
[b] aOR = odds ratios calculated from weighed logistic regression models adjusted for gender, age, race/ethnicity and household income, compared to the ASD- and LD-unaffected group.
[c] CAM = complementary and alternative medicine

CHAPTER 3:

POPULATION PREVALENCE AND ASSOCIATION OF EARLY-LIFE RISK FACTORS FOR NEURODEVELOPMENTAL DISORDERS

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ABSTRACT

Objective: To assess overlap and specificity of pre-, peri-, and neonatal risk factors for ASD and LD in a nationally representative, population-based sample of US adolescents.

Methods: The sample included adolescents aged 13-18 years who participated in the National Comorbidity Survey – Adolescent supplement (NCS-A) whose parents completed a questionnaire concerning their child's lifetime health and development (N=6,295). ASD, LD and pre/perinatal risk factors were determined by parent report. Demographically adjusted odds ratios (aOR) were calculated for ASD (with or without LD) and LD (without ASD) compared to typically developing adolescents (no ASD or LD). Estimates for neonatal complications were additionally adjusted for preterm birth.

Results: Prenatal exposure to alcohol (ASD: aOR=2.43, p=0.017; LD: aOR=1.42, p=0.039), maternal urinary tract infections (UTI) (ASD: aOR=4.46, p=0.018; LD: aOR=1.67, p=0.036), slow heartbeat (ASD: aOR=6.27, p=0.028; LD: aOR=1.86, p=0.031), and respiratory distress (ASD: aOR=5.85, p=0.020; LD: aOR=2.28, p=0.003) at birth were associated with both ASD and LD. Persistent proteinuria was the only obstetric condition associated with any neurodevelopmental outcome (ASD: aOR=13.13, p=0.005). All neonatal complications ascertained in this sample were associated with ASD (convulsions: aOR=9.23, p=0.008, jaundice: aOR=3.21, p=0.028, requiring oxygen: aOR=2.64, p=0.048, blood transfusion: aOR=7.04, p=0.018), but not LD.

Conclusion: ASD and LD appear to share many pre- and perinatal risk factors; however, certain neonatal complications and persistent proteinuria were specifically associated with ASD. Insults during the pre/perinatal period may adversely affect neurodevelopment broadly. Further research should investigate mechanisms by which

maternal persistent proteinuria during pregnancy and neonatal complications are specifically associated with ASD.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder (NDD) characterized by social and communication deficits as well as restrictive and repetitive interests and behaviors (American Psychiatric Association, 2013). ASD symptoms appear very early in life, typically before 24 months of age, and the diagnosis currently affects up to 1 in 68 children nation-wide (CDC, 2014). It has been shown that over 70% of children with ASD go on to develop a learning disorder (LD) (Schieve et al., 2012), a childhood-onset NDD characterized by persistent difficulty in learning academic skills, such as reading, writing, and mathematical reasoning, affecting anywhere from 5-10% of school-age children (Altarac & Saroha, 2007). Both ASD and LD result in varying degrees of lifelong impairment in social, occupational, and daily functioning (American Psychiatric Association, 2013). The causes of these highly comorbid disorders remain unknown, but it has been proposed that the joint influence of genetic and environmental factors may enhance the risk of a range of neurodevelopmental conditions, including ASD.(Hallmayer et al., 2011; Stoltenberg et al., 2010; Tordjman et al., 2014) While progress has been made in identifying independent and additive effects of genetic loci associated with ASD (Gaugler et al., 2014), there is substantial evidence for the etiologic role of environmental risk factors as well. Pre- and perinatal exposures are of particular interest because of the growing evidence for their role in several neuropsychiatric conditions, particularly neurodevelopmental disorders such as ASD and schizophrenia.

Evidence for a range of pre- and perinatal risk factors for ASD and related conditions (Ornoy, Weinstein-Fudim, & Ergaz, 2015; Schieve et al., 2014) as well as general neurodevelopmental problems(Tamaru et al., 2011) from community samples indicate that the period of environmental risk begins in utero for these early-onset conditions. To date, implicated risk factors include health and lifestyle factors during pregnancy (Croen, Grether, Yoshida, Odouli, & Hendrick, 2011; El Marroun, White, van der Knaap, et al., 2014; Krakowiak et al., 2012; Polo-Kantola et al., 2014; Visser et al., 2013; Zerbo, Iosif, et al., 2013; Zerbo, Qian, et al., 2013), obstetric and labor complications (Mamidala et al., 2013; Polo-Kantola et al., 2014; Schieve et al., 2014), perinatal birth outcomes (Lampi et al., 2012; Schieve et al., 2010; Schieve, Clayton, Durkin, Wingate, & Drews-Botsch, 2015; Schieve et al., 2014), and neonatal complications (Froehlich-Santino et al., 2014; Mamidala et al., 2013). However, findings are often inconsistent across studies for a given risk factor (Atladottir, Henriksen, Schendel, & Parner, 2012; Clements et al., 2014; El Marroun, White, Verhulst, & Tiemeier, 2014; El Marroun, White, van der Knaap, et al., 2014; Lee et al., 2012; Lyall, Schmidt, & Hertz-Picciotto, 2014). Moreover, many of these studies have focused on a single risk factor, or one particular window of exposure.

Given the high co-occurrence of ASD with other NDDs, previous studies have investigated the potential for shared or specific risk factors, but much of this work has focused on the potential link between ASD and intellectual disability (ID) (Langridge et al., 2013; Schieve et al., 2015). A recent study evaluating perinatal risk factors for ASD and ID found that many risk factors, such as preterm birth and low birth weight, were associated with both ASD and ID; stronger associations emerged for the children with ID

only, while associations were of similar strength for ASD groups, regardless of the presence of co-occurring ID (Schieve et al., 2015). However, little is known about specificity of prenatal ASD risk factors in the context of broader NDD phenotypes, particularly LD.

Here, we aim to investigate the specificity and commonality of prenatal, perinatal, and neonatal risk factors for ASD and LD using a nationally representative sample of adolescents age 13-18 the National Comorbidity Survey Adolescents Supplement (NCS-A) across a comprehensive range of exposures categories, including maternal characteristics during preconception/pregnancy, obstetric complications during pregnancy, birth outcomes, and neonatal complications. We report prevalence of parent-reported risk factors among adolescents with ASD (with or without co-occurring LD) and LD (without co-occurring ASD). Additionally, we assess demographically adjusted odds of ASD and LD in the presence of such risk factors.

METHODS

Sample

The National Comorbidity Survey Adolescent Supplement (NCS-A) is a nationally representative household survey of adolescent mental health in the Unites States. Recruitment methods, sampling and weighting strategies have been described in detail elsewhere (Kessler et al., 2009). Adolescents aged 13-17 years were recruited via either household or school-based sampling schemes. Professional interviewers at the Survey Research Center at the Institute for Social Research at the University of Michigan administered in-person, computer-assisted personal interviews in adolescents' homes

between February 2001 and January 2004. While adolescents were being interviewed, one parent/guardian was asked to complete a self-administered questionnaire, the Parent Self-Administered Questionnaire (PSAQ), consisting of questions pertaining to the adolescent's medical and developmental history as well as parent and household characteristics. Informed assent and consent was obtained from each participating adolescent and his/her parent/guardian. Human Subjects Committees at the Harvard Medical School and University of Michigan approved recruitment and consent procedures.

Neurodevelopmental Disorders

Autism spectrum disorder (ASD) and learning disorder (LD) were the primary outcomes of interest for this analysis. Presence of ASD and LD were evaluated by parent response to a single yes/no question on in the PSAQ for each condition: "Has [the adolescent] ever had […] developmental disorders (such as autism, Asperger's, or pervasive developmental disorder)?" and "Has [the adolescent] ever had […] learning disability?"

Early-Life Risk Factors

Pre- and perinatal exposures were assessed by parent response to the Birth and Early Development section of the PSAQ. The respondent answered questions regarding the biological mother and father's age at conception, maternal lifestyle behaviors and obstetric complications during pregnancy, birth weight, gestational age, and other birth and neonatal outcomes. Advanced maternal and paternal age was defined as $\geq$ 35 years and $\geq$ 40 years of age, respectively. Preterm birth was defined as < 37 weeks. Low birth

weight was defined as <2500g. Prenatal smoking, alcohol, non-prescription drugs, and caffeine exposure were defined by a response of anything greater than "never" on a 5-point frequency scale. Maternal infections (rubella, urinary tract infection (UTI)), accident/injury, and obstetric complications ("gestational diabetes", "hypertension", "persistent proteinuria", "toxemia" (preeclampsia), "severe nausea and vomiting", "severe anemia", and "placenta problems") were defined as a positive response to the question: "During her pregnancy with this adolescent, did his/her mother have any of the following conditions?" neonatal complications ("blue at birth" (cyanosis), "slow heartbeat", "did not breathe at first" (respiratory distress), "convulsions", "jaundice", "required oxygen", "blood transfusion") were defined as a positive response to the question: "Did he/she have any of the following problems at birth?"

Statistical Analysis

Analyses were completed in R version 3.1.1(R Core Team, 2014) using the R "Survey" package.(Lumley, 2014) All reported prevalence estimates and odds ratios were weighted to represent the demographic characteristics of US population of adolescents attending school at the time of recruitment. Methods used to develop appropriates weights are described elsewhere.(Kessler et al., 2009) Survey-weighted multivariate logistic regression was used to compute odds ratios for ASD (with or without LD) and LD (without ASD) risk compared to a reference group of adolescents without ASD or LD. All odds ratios were adjusted for potential socio-demographic confounders including adolescent sex, age, and race/ethnicity, and house hold income. Odds ratios assessing the effect of birth outcomes and neonatal complications were

further adjusted for preterm birth. Additional sensitivity analyses were completed to explore within-ASD risk heterogeneity. For risk factors that were significantly associated with the ASD, the ASD group was partitioned into ASD with LD (ASD + LD) and ASD without LD (ASD – LD) and each compared to a non-ASD/non-LD reference group.

RESULTS

Table 3.1 reports demographic characteristics of the weighted sample by ASD (n = 51) and LD (n = 879) status, with statistical comparisons made to the unaffected (non-NDD) group (n = 5366). Both NDD-affected groups were predominantly male compared to the non-NDD group: ASD: 85.5% v. 48.2%, $X^2_1 = 15.50$, $p < 0.001$; LD-only: 66.7% v. 48.2%, $X^2_1 = 29.45$, $p < 0.001$. The ASD group also differed significantly from the non-NDD group on distribution of race/ethnicity (X^2_3=7.32, $p < 0.001$). The LD-only group differed significantly from the non-NDD group in terms of lower household income (X^2_3=6.78, p=0.001) and non-intact parental marital status (X^2_3=3.79, p=0.02). The NDD subgroups did not differ from the non-NDD group in mean adolescent age, parent educational status, English spoken in the home, geographic region, or urban environment. Additional comparisons of number of siblings and birth order revealed no differences between any NDD group and the non-NDD group. Sex, age, household income, and parent education were included as covariates in subsequent analyses.

Maternal characteristics during pregnancy as well as advanced paternal age at conception are reported in Table 3.2. Advanced maternal age (≥ 35 years) was associated with increased odds for LD (aOR = 1.67, p = 0.027), but not ASD. Alcohol consumption (ASD: aOR = 2.43, p = 0.017; LD: aOR = 1.42, p = 0.039), and urinary tract infection

(UTI) (ASD: aOR = 4.46, p = 0.018; LD: aOR = 1.67, p = 0.036) during pregnancy were associated with both ASD and LD. Rubella infection (aOR = 123.57, p<0.001) and injury (aOR = 8.01, p = 0.012) during pregnancy were associated with increased ASD risk, but not with LD risk.

Table 3.3 presents prevalence and adjusted odds ratios related to obstetric complications. Persistent proteinuria (aOR = 13.13, p = 0.005) was significantly associated with increased ASD risk, but not LD, while severe nausea and vomiting was associated with increased risk for LD (aOR = 1.68, p = 0.009), but not ASD. All other pregnancy-related health problems were not associated with ASD or LD.

Preterm birth was associated with more than 3-fold risk for ASD (aOR = 3.57, p = 0.018) and more than 2-fold (aOR = 2.33, p = 0.001) risk for LD. Low birth weight (< 2500g) was associated with LD (aOR = 2.52, p = 0.004), but not ASD, however this association was attenuated after controlling for preterm birth. Table 3.4 presents prevalence and adjusted odds ratios related to birth outcomes of the child, additionally adjusted for preterm birth. Slow heartbeat (ASD: aOR = 6.27, p = 0.28; LD: aOR = 1.86, p = 0.31) and respiratory distress (ASD: aOR = 5.85, p = 0.020; LD: aOR = 2.28, p = 0.003) were associated with both ASD and LD, while cyanosis at birth (aOR = 1.90, p = 0.021) was specifically associated with a nearly 2-fold increase in odds for LD.

Prevalence and adjusted odds ratios related to neonatal complications are presented in Table 3.5. Initially, after controlling for demographics only, blood transfusion and requiring oxygen was associated with both ASD and LD, while convulsions and jaundice were specifically associated with ASD. However, after controlling for demographics and preterm birth, all neonatal complications – convulsions

(aOR = 9.23, p = 0.008), jaundice (aOR = 3.12, p = 0.028), requirement of oxygen (aOR = 2.64, p = 0.048), and blood transfusion (aOR = 7.04, p = 0.018) – remained significantly associated with ASD, but none remained for LD (Table 3.5).

Risk factors significantly associated with ASD were assessed for heterogeneity, separately comparing ASD with and without concurrent LD to the non-NDD group. Prenatal alcohol exposure (aOR = 6.11, p = 0.002) and persistent proteinuria (aOR = 28.67, p = 0.004) were specifically associated with ASD without LD, but not ASD with LD. Injury during pregnancy (aOR = 12.89, p = 0.004), preterm birth (aOR = 5.43, p = 0.006), slow heartbeat (aOR = 10.37, p = 0.008), delayed respiration (aOR = 9.92, p = 0.005), convulsions (aOR = 13.39, p = 0.003), jaundice (aOR = 3.82, p = 0.027), oxygen requirement (aOR = 4.05, p = 0.023), and blood transfusion (aOR = 9.50, p = 0.011) remained positively associated with ASD with LD, but not ASD without LD.

DISCUSSION

We used retrospective parent-reported pre- and perinatal data from a nationally representative sample of adolescents to identify potential sources of heterogeneity of early-life risk factors for NDDs. Exposure windows spanning pre-conception through the neonatal period were assessed. We found that many of these risk factors were shared across NDDs (ASD and LD), but generally showing larger effect sizes in the more severe ASD group. However, persistent proteinuria during pregnancy and neonatal complications were associated with ASD, and not LD. Conversely, advanced maternal age, severe nausea and vomiting during pregnancy, and, after controlling for preterm birth, cyanosis was specifically associated with LD.

ASD specific risk factors

Rubella infection, accident/injury, and persistent proteinuria during pregnancy were exposures among the prenatal exposure window specifically associated with ASD. Maternal rubella infection has been linked to ASD previously(Ornoy et al., 2015), however due to the very low prevalence of rubella exposure in this sample, the very large effect size should be interpreted with caution. Persistent proteinuria was the only obstetric factor related to ASD. A meta-analysis also suggests that other obstetric health concerns such as maternal hypertension, pre-eclampsia, edema, etc. are not associated with ASD; however, they also found no evidence for an association between proteinuria and ASD, although the authors caution largely inconsistent results from individual studies (Gardener, Spiegelman, & Buka, 2009). However, more recently, maternal edema has been linked to ASD (Zhang et al., 2010) and social and repetitive behavior domains as measured by the ADI-R (Wallace, Anderson, & Dubrow, 2008). Persistent proteinuria, another symptom of pre-eclampsia, has been shown to significantly affect placental perfusion, decreased when occurring early in pregnancy and increased perfusion in late pregnancy.(Sohlberg et al., 2014) Thus, depending on timing, the link between proteinuria and ASD may be a consequence of decreased cerebral blood flow in the fetus. It should be noted that we were unable to assessing timing of proteinuria onset. After controlling for preterm birth, requirement of oxygen and blood transfusion remained associated with ASD, but not LD. While it is intriguing that every neonatal complication evaluated in this study showed specificity to ASD, it is important to note that these neonatal complications could be a result of larger underlying health problems associated with ASD caused by an earlier exposure, rather than a root cause of ASD symptoms.

Nonetheless, identification of ASD-specific-risk factors, whether causes or mediators, may elucidate etiologic pathways related to ASD-specific symptoms or traits.

LD specific risk factors

Mothers of advanced maternal age (≥ 35 years) at birth had nearly 70% higher odds of reporting LD of their adolescent, and no increase in odds of ASD when compared to the ASD- and LD-unaffected group. Interestingly, advanced maternal age is often reported as a risk factor for ASD (Gardener et al., 2009; Guinchat et al., 2012; Mamidala et al., 2013), but our study did not confirm this association. However, others have found an association with advanced maternal age only with ASD cases on the milder end of the autism spectrum, including Asperger syndrome and pervasive developmental disorder NOS (Lampi et al., 2013), and we did not have the ability to split our ASD group by severity. Severe nausea and vomiting was the only obstetric factor that was specifically associated with LD. While nausea and vomiting during pregnancy is a common occurrence, the *absence* of nausea and vomiting during the first trimester has been linked to hypothyroidism (Forbes, 2014) during pregnancy and has also been linked ASD in the child (Brown, Jones, MacKewn, & Plank, 2008). This specific association to LD (and not ASD) could indicate a specific association between the lack of pregnancy sickness and ASD. However, our questionnaire specifically asked about "severe" nausea and vomiting, which is highly subjective to participant interpretation. While low birth weight initially showed a significant association with LD, cyanosis was the only birth outcome that showed LD-specific associations after controlling for preterm birth. Low Apgar scores (Andrews, Goldberg, Wellen, Pittman, & Struening, 1995) and a combination of low Apgar and shared genes have been linked to LD (Garanty-Bogacka, Wieczorek, &

Syrenicz, 1998). Further work should explore whether these LD-specific associations may indicate a separate etiology for the milder LD phenotype

Non-specific risk factors

Alcohol consumption and UTI during pregnancy were associated with both ASD and LD. A recent review of the relationship between prenatal toxicant exposure and ASD postulates that maternal infection and exposure to toxicants, such as alcohol, causes a maternal inflammatory response leading to micronutrient deficiency, thereby disrupting fetal brain development (Nuttall, 2015). We did not assess extent of alcohol consumption or severity of UTI infection, but both appear to be non-specific insults with potential varying degrees of exposure intensity, possibly affecting a continuum of neurodevelopmental outcomes by extent of exposure. Preterm birth was also associated with NDDs broadly. After controlling for preterm birth, slow heartbeat, and respiratory distress were also broadly associated with both ASD and LD. These results support previous work linking preterm birth (Guinchat et al., 2012; Lampi et al., 2012; Meldrum et al., 2013) and low Apgar scores several NDDs, including ASD (Froehlich-Santino et al., 2014; Guinchat et al., 2012; Mamidala et al., 2013; Schieve et al., 2015). An assessment of perinatal risk factors associated with ASD and ID recently reported a stronger association between preterm birth and the more severe ID phenotype in comparison to the weaker, but still significant ASD association (Schieve et al., 2015). The current findings are in line with this concept, where we see stronger association between preterm birth and ASD compared to the lower, yet still significant, LD effect size.

Limitations

While over 6,000 adolescents were included in the current study, the low prevalence of ASD resulted in a small ASD-affected NDD subgroup, thereby widening confidence intervals associated with those estimates. However, this results from the nature of using nationally representative survey data, and we believe the benefit of a population-based sample free of clinical ascertainment bias adds substantial value to literature in the field, even with methodological limitations. Furthermore, prevalence estimates of both ASD and LD obtained from the current study are similar to what is expected for the participants' age at the time of recruitment (Altarac & Saroha, 2007; CDC, 2007). Because parents were asked to report on pre/perinatal exposures and events of their adolescent children, results may be affected by recall bias, where parents of children with ASD are may be more likely to remember or over-endorse prenatal and neonatal complications. However, several findings in this study reflect what has been found in previous ASD and LD literature, lending some validation to our exposure measures.

Strengths

First, we use a nationally representative sample where we do not expect a disproportionate amount of severe cases that could result from clinical ascertainment. Second, we are able to report comparison with an LD-only affected group to identify specificity of ASD-related risk factors from a less sever NDD phenotype. Previous work has focused on differentiating ASD risk factors from those of ID, a more severe NDD phenotype. Third, we assess a wide range of early-life risk factors spanning the entire prenatal through neonatal period, which allowed us to assess potential differences in exposure timing between groups.

Conclusion

This work has both etiologic and clinical relevance. ASD-specific risk factors may be etiologically related in ASD-specific traits, while shared risk factors may be etiologically linked to general perturbations in neurodevelopment. We found that insults during the pre/perinatal period may adversely affect neurodevelopment broadly. Future research should investigate mechanisms by which prenatal persistent proteinuria and neonatal complications are specifically associated with ASD. Additionally, identified early-life risk factors could be analyzed in combination with genetic data to aid in completing full picture of NDD etiology. Given the high comorbidity among NDDs, future research should further investigate specificity and overlap concerning not only diagnostic criteria, but also individual symptom/trait-related phenomenology of NDDs. Clinically, obstetric complications such as proteinuria, birth outcomes, and neonatal complications may warrant timely developmental surveillance which could lead to earlier intervention, and therefore, better prognosis. Further work should be done to understand where these risk factors lie on the causal pathway. Perhaps most importantly, some of these pre/perinatal exposures are malleable risk factors that could potentially aid in prevention.

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Table 3.1. Demographic characteristics of NCS-A adolescents age 13-18 years by ASD and LD status, compared to adolescents without either disorder [a]

Demographic Characteristics	Autism Spectrum Disorder (N = 51)		Learning Disorder Only (N = 879)		None (N = 5,366)
	% (SE)	X^2, p	% (SE)	X^2, p	% (SE)
Gender		15.50, <0.001		29.45, <0.001	
male	85.5 (5.0)		66.7 (3.1)		48.2 (1.3)
female	14.5 (5.0)		33.3 (3.1)		51.8 (1.3)
Age (mean)		-0.34[b], 0.74		1.01[b], 0.32	
	15.1 (0.33)		15.3 (0.12)		15.2 (0.07)
Race/Ethnicity		7.32, <0.001		0.41, 0.75	
Hispanic	3.9 (2.0)		12.9 (1.9)		14.7 (1.5)
Black	26.6 (7.4)		16.0 (2.0)		14.4 (1.1)
Other	14.4 (2.7)		6.5 (2.7)		4.7 (0.7)
White	55.1 (8.1)		64.5 (4.2)		66.1 (1.9)
Household Income [c]		1.45, 0.24		6.78, 0.001	
PIR ≤ 1.5	30.2 (9.9)		19.6 (2.5)		13.2 (1.1)
1.5 < PIR ≤ 3	22.0 (8.9)		20.2 (2.7)		19.2 (0.8)
3 < PIR ≤ 6	29.7 (8.6)		24.7 (2.5)		34.1 (1.2)
PIR > 6	18.1 (6.4)		35.6 (3.0)		33.6 (1.6)
Parent Marital Status		1.23, 0.31		3.79, 0.02	
Married/ cohabiting	45.6 (10.1)		62.3 (2.3)		69.7 (1.4)
Previously married	19.4 (8.5)		19.3 (2.1)		15.7 (0.7)
Never married	8.1 (4.6)		3.9 (1.0)		4.4 (0.6)
Unknown	26.8 (9.7)		14.6 (1.9)		10.3 (0.9)
Parent Education		1.82, 0.16		1.73, 0.18	
Less than high school	29.0 (10.4)		16.6 (2.2)		11.2 (0.9)
High school graduate	20.2 (8.1)		30.6 (2.8)		29.0 (1.2)
Some college	11.0 (4.3)		20.7 (2.1)		21.7 (1.0)
College graduate	39.8 (9.8)		32.2 (3.8)		38.1 (1.6)
English spoken at home		0.22, 0.64		3.81, 0.06	
	85.5 (8.9)		86.1 (2.2)		81.3 (0.9)
Geographic region		0.37, 0.78		1.61, 0.20	
Northeast	25.9 (8.4)		17.0 (2.2)		18.4 (1.8)
Midwest	23.9 (8.5)		21.5 (2.4)		23.5 (2.1)
South	30.9 (7.5)		36.1 (3.9)		36.1 (2.6)
West	19.2 (9.0)		25.4 (2.4)		22.0 (2.0)
Urban environment		1.95, 0.15		2.03, 0.14	
Metropolitan	32.0 (8.9)		41.1 (3.7)		46.7 (2.4)
Rural	32.7 (9.4)		44.9 (3.9)		38.7 (3.2)
Other	35.4 (9.8)		14.0 (2.8)		14.5 (2.3)

[a] Percentages are weighted to the general population of US adolescents based on 51 adolescents with parent-reported ASD, 879 with LD, and 5,366 adolescents without ASD or LD

[b] t-value

[c] Household income as measured by the income-to-poverty ratio (PIR)

Table 3.2. Prevalence of reported maternal characteristics during pregnancy and associated odds of reporting ASD and LD in the adolescent [a]

	Autism Spectrum Disorder (N = 51)	Learning Disorder Only (N = 879)	None (N = 5,366)	Autism Spectrum Disorder		Learning Disorder Only	
	% (se)	% (se)	% (se)	aOR [b] (95% CI)	p-value	aOR (95% CI)	p-value
Advanced maternal age (≥35)	2.5 (1.8)	11.4 (1.8)	7.3 (0.7)	0.34 (0.07, 1.60)	0.181	**1.67 (1.08, 2.59)**	**0.027**
Advanced paternal age (≥40)	3.8 (2.0)	5.8 (1.1)	6.0 (0.6)	0.59 (0.17, 2.01)	0.406	0.98 (0.66, 1.45)	0.915
Smoking	26.2 (9.2)	24.5 (2.4)	21.0 (0.5)	1.23 (0.45, 3.36)	0.692	1.18 (0.89, 1.56)	0.251
Alcohol	32.1 (7.6)	22.9 (2.8)	17.9 (0.1)	**2.43 (1.21, 4.9)**	**0.017**	**1.42 (1.03, 1.97)**	**0.039**
Non-prescription drug	11.6 (7.2)	5.9 (1.1)	3.9 (0.6)	2.88 (0.71, 11.69)	0.147	1.47 (0.97, 2.24)	0.079
Caffeine	46.0 (11.3)	67.5 (2.1)	64.2 (0.4)	0.44 (0.16, 1.21)	0.121	1.17 (0.92, 1.49)	0.206
Rubella	8.7 (4.8)	0.1 (0.1)	0.1 (2.3)	**123.57 (25.86, 590.45)**	**<0.001**	2.08 (0.31, 13.81)	0.453
Urinary tract infection	22.5 (9.3)	9.6 (1.7)	6.2 (0.4)	**4.46 (1.37, 14.51)**	**0.018**	**1.67 (1.05, 2.65)**	**0.036**
Accident/injury	11.2 (7.0)	2.5 (1.0)	1.5 (0.2)	**8.01 (1.71, 37.49)**	**0.012**	1.78 (0.76, 4.14)	0.190

[a] Percentages and odds ratios are weighted to the general population of US adolescents based on 51 adolescents with parent-reported ASD, 879 with LD, and 5,366 without ASD or LD

[b] aOR= adjusted odds ratio; all aORs are adjusted for sex, age, race/ethnicity, and household income

Table 3.3. Prevalence of reported obstetric complications and associated odds of reporting ASD and LD in the adolescent [a]

	Autism Spectrum Disorder (N = 51)	Learning Disorder Only (N = 879)	None (N = 5,366)	Autism Spectrum Disorder		Learning Disorder Only	
	% (se)	% (se)	% (se)	aOR [b] (95% CI)	p-value	aOR (95% CI)	p-value
Gestational diabetes	9.5 (7.0)	6.4 (1.9)	4.3 (17.6)	2.27 (0.4, 12.97)	0.362	1.59 (0.83, 3.04)	0.166
Hypertension	14.1 (7.3)	10.3 (1.4)	7.9 (19.0)	2.08 (0.63, 6.82)	0.237	1.40 (0.95, 2.05)	0.094
Persistent proteinuria	8.7 (6.4)	0.3 (0.2)	0.5 (0.2)	**13.13 (2.4, 71.88)**	**0.005**	0.51 (0.1, 2.68)	0.435
Toxemia	6.5 (3.7)	5.6 (1.3)	5.1 (0.7)	1.45 (0.43, 4.87)	0.548	1.15 (0.66, 2.04)	0.622
Severe nausea and vomiting	11.3 (4.9)	12.4 (1.9)	7.9 (0.7)	1.49 (0.58, 3.83)	0.408	**1.68 (1.16, 2.44)**	**0.009**
Severe anemia	5.4 (3.9)	4.4 (1.1)	2.9 (0.4)	2.11 (0.47, 9.5)	0.336	1.65 (0.92, 2.99)	0.103
Placenta problems	3.9 (3.0)	3.4 (1.0)	2.1 (0.4)	1.62 (0.3, 8.76)	0.580	1.66 (0.88, 3.12)	0.125

[a] Percentages and odds ratios are weighted to the general population of US adolescents based on 51 adolescents with parent-reported ASD, 879 with LD, and 5,366 without ASD or LD

[b] aOR= adjusted odds ratio; all aORs are adjusted for sex, age, race/ethnicity, and household income

Table 3.4. Prevalence of reported birth outcomes and associated odds of reporting ASD and LD [a]

	Autism Spectrum Disorder (N = 51)	Learning Disorder Only (N = 879)	None (N = 5,366)	Autism Spectrum Disorder		Learning Disorder Only	
	% (se)	% (se)	% (se)	aOR (95% CI)	p-value	aOR (95% CI)	p-value
Low birth weight (< 2500g)	10.5 (5.6)	10.1 (2.1)	4.6 (0.4)	0.53 (0.09, 3.29)	0.500	2.09 (0.96, 4.52)	0.070
Pre-term birth (<37 weeks) [c]	15.4 (6.2)	9.9 (1.8)	4.5 (0.4)	**3.57 (1.30, 9.77)**	**0.018**	**2.33 (1.44, 3.78)**	**0.001**
Caesarian section	31.7 (9.4)	28.4 (2.9)	25.3 (0.9)	1.60 (0.66, 3.9)	0.308	1.16 (0.84, 1.60)	0.370
Cyanosis at birth	13.4 (5.7)	7.7 (1.8)	3.9 (0.5)	2.28 (0.79, 6.63)	0.138	**1.90 (1.13, 3.21)**	**0.021**
Slow heartbeat	15.9 (10.4)	5.9 (1.3)	2.6 (0.4)	**6.27 (1.30, 30.18)**	**0.028**	**1.86 (1.08, 3.2)**	**0.031**
Respiratory distress	17.0 (10.3)	8.9 (1.9)	3.5 (0.4)	**5.85 (1.41, 24.35)**	**0.020**	**2.28 (1.36, 3.82)**	**0.003**

[a] Percentages and odds ratios are weighted to the general population of US adolescents based on 51 adolescents with parent-reported ASD, 879 with LD, and 5,366 without ASD or LD

[b] aOR= adjusted odds ratio; all aORs are adjusted for sex, age, race/ethnicity, household income, and preterm birth

[c] preterm birth is not adjusted for preterm birth

Table 3.5. Prevalence of reported neonatal complications and associated odds of reporting ASD and LD [a]

	Autism Spectrum Disorder (N = 51)	Learning Disorder Only (N = 879)	None (N = 5,366)	Autism Spectrum Disorder		Learning Disorder Only	
	% (se)	% (se)	% (se)	aOR [b] (95% CI)	p-value	aOR (95% CI)	p-value
Convulsions	3.3 (2.6)	0.9 (0.8)	0.4(0.1)	**9.23 (1.96, 43.49)**	**0.008**	2.66 (0.32, 22.05)	0.371
Jaundice	33.3 (10.4)	16.6 (1.9)	13.0 (0.6)	**3.21 (1.18, 8.68)**	**0.028**	1.15 (0.81, 1.63)	0.447
Required oxygen	20.1 (7.6)	11.6 (2.4)	5.2 (0.6)	**2.64 (1.04, 6.67)**	**0.048**	1.77 (0.95, 3.28)	0.080
Blood transfusion	10.9 (5.6)	2.2 (0.9)	0.7(0.2)	**7.04 (1.5, 33.1)**	**0.018**	2.03 (0.58, 7.12)	0.275

[a] Percentages and odds ratios are weighted to the general population of US adolescents based on 51 adolescents with parent-reported ASD, 879 with LD, and 5,366 without ASD or LD

[b] aOR= adjusted odds ratio; all aORs are adjusted for sex, age, race/ethnicity, household income, and preterm birth

CHAPTER 4:

GENETIC DETERMINANTS OF COGNITIVE DEVELOPMENTAL TRAJECTORIES IN INFANTS AT HIGH-RISK FOR AUTISM SPECTRUM DISORDER

ABSTRACT

Background: Autism spectrum disorder (ASD) is a heritable, heterogeneous neurodevelopmental disorder affecting social communication and motor stereotypies. Younger siblings of individuals with ASD are at elevated risk for developing ASD and related traits, compared to the general population, and have been an important group for examining early signs and symptoms of ASD. Early developmental trajectories of communication, social, and motor abilities are a source of phenotypic heterogeneity in ASD and may serve as a useful phenotype for genetic analyses.

Objective: The aim of this study is to identify common ASD-related genetic variants associated with differential trajectories of cognitive and motor development among infant high-risk ASD infant siblings.

Methods: Infant siblings of ASD-affected children were enrolled during pregnancy in the Early Longitudinal Autism Investigation (n=231) and just after birth in the Infant Brain Imaging Study (n=382). Latent subgroups of cognitive and motor development were estimated based on longitudinal Mullen Scales of Early Learning (MSEL) (at 6, 12, 24, and 36 months of age) using latent class growth analysis (LCGA). A 3-step latent class regression method was used to test for association of ASD polygenic risk score (PRS) at varying inclusion thresholds ($p<1x10^{-3}$, $p<1x10^{-4}$, $p<1x10^{-5}$) and association with each of 4,257 SNPs within 47 ASD candidate genes among EARLI high-risk siblings for whom genotype data were available (n=198).

Results: Using EARLI and IBIS longitudinal data, three latent classes were identified labeled “normative”, “intermediate”, and “declining” based on MSEL patterns. ASD PRS (threshold = $p<1x10^{-3}$) was marginally associated with the “declining” class

(standardized: OR= 1.66, p = 0.083) when compared to the "normative" class and was significantly associated with ASD diagnosis (standardized: OR=1.64, p=0.032, quartiled: OR=7.72, p=0.013). No candidate SNPs reached Bonferroni significance ($1.2x10^{-5}$), but chr3:2928197 in *CNTN4* and rs10265509 in *CNTNAP2* on chromosome 7 reached suggestive significance ($p < 1x10^{-3}$) comparing the "intermediate" to the "normative" class.

Conclusion: Latent class structure was consistent with previous literature. Liberal PRS of common genetic variation associated with ASD was marginally associated with declining cognitive trajectory and was significantly associated with ASD diagnosis at 36 months. The candidate genes most strongly associated with class memberships share roles in modulating neuronal cell interactions and are associated with a broad range of neuropsychiatric phenotypes beyond ASD.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder defined by social and communication deficits as well as restrictive and repetitive interests and behaviors (American Psychiatric Association, 2013). Heritability studies suggest that a substantial portion of phenotypic variance in ASD may be due to genetic factors (Colvert et al., 2015; Hallmayer et al., 2011; Sandin et al., 2014), with nearly 50% of this genetic risk due to common genetic variation (Gaugler et al., 2014; Klei et al., 2012). However, genome-wide association studies (GWAS) designed to target common polymorphisms have identified only a few genome-wide significant risk alleles, most of which fail to replicate (Anney et al., 2012; Anney et al., 2010; Curran et al., 2011; Liu & Takumi, 2014; K. Wang et al., 2009; Weiss, Arking, Daly, & Chakravarti, 2009). This lack of identified common variants could be due to a lack of sufficient statistical power, and thus large mega-analyses of GWAS studies are underway ("Psychiatric Genomics Consortium,"). However, it could also be that diagnostic phenotypes are not best suited for etiologic discovery due to phenotypic heterogeneity *within* diagnostic boundaries and associated traits that *cross* diagnostic boundaries. Such, muddled phenotypes present a challenge in detecting potential genetic associations, particularly in common variant studies where effect sizes are likely to be small.

ASD symptom onset occurs in infancy and progresses through toddlerhood (Jones, Gliga, Bedford, Charman, & Johnson, 2014; Ozonoff et al., 2010; Rozga et al., 2011). But, symptom onset and course differ across cases, and are major sources of phenotypic heterogeneity in ASD. These differential trajectories begin before diagnosis (Landa, Gross, Stuart, & Faherty, 2013) and continue throughout childhood (Fountain,

Winter, & Bearman, 2012; Szatmari et al., 2015). Longitudinal studies assessing cognitive and motor deficits have found heterogeneous trajectories of cognitive and motor abilities among infants and toddlers at high risk for developing ASD (Estes et al., 2015; Landa, Gross, Stuart, & Bauman, 2012). Understanding specific developmental pathways associated with ASD may inform our understanding of underlying mechanisms involved in ASD etiology.

Moreover, examining data across a continuous spectrum of ASD-related traits, such as cognitive and motor ability, may provide insight for genetic studies of ASD, and neurodevelopment generally. While attempts at splitting an ASD sample by IQ, ASD severity, and ASD symptom profiles to account for genetic heterogeneity have not yielded better results than using the full ASD sample in simplex family cases(Chaste et al., 2014), there is evidence that examining traits across ASD and other disorders may be fruitful. ASD genetic risk variants may influence developmental traits on a continuum and affect many neurodevelopmental disorders beyond ASD (Coe, Girirajan, & Eichler, 2012; Lichtenstein, Carlstrom, Rastam, Gillberg, & Anckarsater, 2010), as well as affect these traits in neuro-typical individuals (Robinson et al., 2016).

Younger siblings of children with ASD are nearly 20 times more likely to develop ASD compared to the general population (Ozonoff et al., 2011). Therefore, prospective cohort studies of infant siblings at high risk for developing ASD allow direct observation of patterns of early development from infancy, before a diagnosis is made. Here, we use two high-risk infant studies, the Early Autism Risk Longitudinal Investigation (EARLI) and the Infant Brain Imaging Study (IBIS) to characterize very early cognitive and motor developmental trajectories and identify common ASD-related genetic variants associated

with differential trajectories. First, we identify latent subgroups of cognitive and motor development as measured longitudinally from six to 36 months of age by the Mullen Scales of Early Learning (MSEL) using latent class growth analysis (LCGA). Second, we assess association of an ASD polygenic risk score (PRS) with trajectory class. Third, we test for association with specific common variants in ASD candidate genes with trajectory class.

METHODS

Sample

The EARLI-IBIS combined cohort is a collaborative effort between the Early Autism Risk Longitudinal Investigation (EARLI) and the Infant Brain Imaging Study (IBIS) designed to study genetic predictors of ASD and ASD-related phenotypes among high risk infant siblings of children with ASD. Recruitment and data collection methods are described in detail elsewhere for EARLI (Newschaffer et al., 2012) and IBIS (Estes et al., 2015; Hazlett et al., 2012). EARLI (n=231) and IBIS (n=382) high risk siblings with available MSEL data for at least one of 6, 12, 24, or 36 month visits were included in the latent class growth analysis (LCGA). EARLI high-risk siblings from the LCGA who had available genotype data were included in the genetic association analyses (n=198).

Latent Trajectory Model and Latent Class Regression Method

The MSEL (Mullen, 1995) is a clinician-administered standardized (m=50, sd=10) assessment which measures five domains of early cognitive (expressive language, receptive language, and visual reception) and motor (fine motor and gross motor) development. EARLI-IBIS high-risk sibling participants were administered the MSEL at

6, 12, 24, and 36 months of age. The gross motor scale is not applicable at 36 months. Domain scores for each applicable visit were modeled simultaneously in a parallel-process latent class growth analysis (PP-LCGA) model to obtain latent classes of cognitive development trajectory. A quadratic term was added to the model based on lower BIC statistic yielded from a quadratic 1-class model compared to a linear 1-class model. Next, a series of 1- through 5-class PP-LCGA models were estimated with variance of intercepts, slopes, and quadratic terms constrained to zero, assuming homogeneity of individual growth trajectories within each class. After considering information criteria statistics and theoretical plausibility, a final model was used as the dependent variable in latent class regression analyses with various genetic markers as independent variables.

Genotype quality control and imputation

Genotype quality control procedures were implemented using PLINK version 1.9 (Chang et al., 2015; Purcell & Chang). DNA samples from EARLI participants were genotyped along with 18 HapMap control samples on the Human Omni5 plus Exome genotyping array (Illumina, San Diego, CA) at the Center for Inherited Disease Research at Johns Hopkins University, generating data on 4,641,218 SNPs. The concordance rate between duplicated HapMap samples and five EARLI blind technical duplicates was 99.72% and 99.9%, respectively. Samples were filtered out from analysis if missing data at >3% of markers, excess heterozygosity/homozygosity (>4 SD). Markers were removed from further analysis if the genotyping facility reported technical problems (n=94,712), missing genomic location information (n=8,124), missing rate ≥ 5% for high minor allele frequency (MAF) (>5%) SNPs (n=8,902), and missing rate > 1% for low

MAF (< 5%) SNPs (n=65,855). SNPs out of Hardy-Weinberg equilibrium ($p< 1 \times 10^{-7}$) were flagged (n=2,170). Principal components of genetic ancestry were computed using the PLINK --pca function in combination with 1000 Genomes Project (1000GP) (Siva, 2008) samples. EARLI measured genotype data was phased using Shape-IT (Delaneau, Coulonges, & Zagury, 2008) and imputed to the 1000GP data using minimac3 (Fuchsberger, Abecasis, & Hinds, 2015; Howie, Fuchsberger, Stephens, Marchini, & Abecasis, 2012). SNPs with MAF > 1% were retained, leaving a total of 9,377,008 SNPs for analysis.

ASD polygenic risk score

ASD polygenic risk scores (PRS) were calculated using results from the Psychiatric Genomics Consortium (PGC) mega-analysis (n = 10,610) ("Psychiatric Genomics Consortium,"). Score were created using all SNPs achieving specific PGC p-value thresholds: 1×10^{-3}, 1×10^{-4}, 1×10^{-5}. Of the 11,660, 1,096, and 135 PGC SNPs passing these thresholds respectively, 10,859; 1,027; and 127 were available in the EARLI measured and imputed SNP set. These SNPs were further pruned for independence ($r^2 < 0.5$) within a sliding 500kb window, to leave 6605; 504; and 20 SNPs, respectively. Dosages from these remaining independent SNPs were weighted by their respective PGC log odds ratios and summed to calculate three PRS values for each sample.

ASD candidate gene selection

Given that this sample is too small for genetic discovery, we examined a list of known ASD candidate genes to identify which may be related to early life trajectories. We obtained a list of ASD candidate genes defined as “high confidence” or “strong

candidate" genes from the Simons Foundation Autism Research Initiative (SFARI) online database (accessed June 24, 2016) (Basu, Kollu, & Banerjee-Basu, 2009). A total of 64,277 SNPs in our data overlapped with 50 candidate genes. To reduce computational burden, these SNPs were pruned at $r^2 > 0.5$ within a 500kb sliding window, yielding 12,429 SNPs in 48 candidate genes. Further filtering at MAF > 5% among EARLI high-risk siblings, yielded 4,257 SNPs in 47 candidate genes to be used in association analyses described below.

Genetic association analyses

Association between each PRS and ASD status was tested by logistic regression, adjusting for sex and ancestral principal components 1 through 5. Standardized PRS odds ratios represent risk of ASD or latent class membership per 1 standard deviation increase in PRS. Quartiled PRS odds ratios represent risk of ASD or class membership for those in the top quartile of PRS scores compared to the bottom quartile.

A 3-step latent class regression approach was used in order to preserve the class structure across all PRS and SNP comparisons while still obtaining appropriate estimates and standard errors accounting for uncertainty in class assignment (Asparouhov & Muthén, 2013). We performed multinomial regression models that were adjusted for gender and the first 5 principal components of genetic ancestry to reduce potential for confounding by population stratification. For the PRS analyses, each PRS was standardized and change in log odds of class membership was calculated based on 1 standard deviation increase in PRS. For the candidate gene SNP analyses, an additive model was tested, with the minor allele as the risk allele. A Bonferroni significance threshold was set at $p < 1.2 \times 10^{-5}$ to account for multiple comparisons (0.05/4,257).

Suggestive significance was defined as $p < 1x10^{-3}$. For the two genes with the strongest associations to latent class membership, we repeated the latent class regression approach for all SNPs from the EARLI measured or imputed set within those genes and graphed the results using LocusZoom software (Pruim et al., 2010). Additional loci were mapped to these plots based on queries of the NHGRI GWAS catalog (Burdett T; Welter et al., 2014).

PP-LCGA and latent class regression analyses were implemented using MPLUS version 7 (Muthén & Muthén, 1998-2015).

RESULTS

Sample characteristics, including study sample sizes, DSM-IV ASD diagnoses, sex, and mean MSEL domain T scores for EARLI (n=231) and IBIS (n=382) high-risk siblings are reported in Table 4.1. Latent classes of cognitive development trajectory are shown in Figure 4.1. Class proportions and fit statistics for these models are shown in Table 4.2. The 3 class model was chosen as the final model for analysis based on plateauing of various information criteria statistics and theoretical plausibility. Figure 4.2 shows sample MSEL means for each class for 6, 12, 24, and 36 month visits. The gross motor domain is not administered past 33 months of age, so we report only 6, 12, and 24 month visit means. Based on general patterns of MSEL domain scores over time, we labeled the classes "normative", "intermediate", and "declining"; class proportions based on the estimated model were 33%, 46%, and 21%, respectively. Table 4.3 shows the distribution of ASD diagnosis and sex by most-likely class assignment based on posterior probability of class membership estimated by the PP-LCGA model. The "declining" class

had the highest proportion of ASD cases, followed by the "intermediate" class and the "normative" class. The proportion of males in the each class exhibited a similar pattern. Table 4.4 shows PRS associations with latent class membership and ASD diagnostic status. PRS was not statistically significantly associated with class membership or ASD status when using stringent p-value thresholds for PRS membership (e.g. $1x10^{-4}$, $1x10^{-5}$). However, when using liberal thresholds (e.g., $p<1x10^{-3}$), standardized PRS is marginally associated with "declining" class (OR = 1.66, p = 0.083) and significantly associated with ASD (OR = 1.64, p = 0.032). The most notable association is comparing the top versus bottom quartile of PRS by ASD status (OR = 7.72, p = 0.013).

Figure 4.3 shows latent class association p-values for all SNPs located in one of the 47 ASD candidate genes. For both the intermediate (Panel A) and declining (Panel B) classes, no SNPs reached Bonferroni significance. However, for the "intermediate" class, two SNPs reached suggestive significance: chr3:2928197 located in the *CNTN4* gene (OR = 0.23, p=0.001) and rs10265509 in the *CNTNAP2* gene on chromosome 7 (OR = 9.30, p = 0.001). No SNPs in the "declining" class comparison reached suggestive significance. However, more than twice as many SNPs (n=251) reached nominal significance (p<0.05) for the "declining" class (Supplementary Table 4.3) than for the "intermediate" class (n=117) (Supplementary Table 4.1). Table 4.5 and Table 4.6 show the SNPs with the 10 smallest p values for the "intermediate" and "declining" class, respectively. There was no overlap of specific SNPs among these tables, although four genes (*CNTN4*, *CTNND2*, *CNTNAP2,* and *KAT2B*) were in both lists. There was moderate correlation of effect sizes between the "intermediate" and "declining" classes for all candidate gene SNPs tested (r=0.32, $p = 2.2 \times 10^{-16}$) (Figure 4.4). Table 4.7 shows

chromosome, position, gene, and effect size and p-value for the "intermediate" and "declining" groups for the top 10 SNPs based on joint ranking of p-values from both comparison groups. Four SNPs in this group harbored in *GRIN2B* displayed effects of similar direction and magnitude in both comparison groups.

Supplementary Figure 4.1 depicts adjusted odds ratios and 95% confidence intervals for these 10 SNPs to show strength of association and direction of effects. The direction of effect was consistent for all of these SNPs in the two comparison groups; one exception was a SNP harbored in *CNTNAP2*, which showed a protective effect in the "intermediate" class but a risk effect in the "declining" class. Gene-based results are ranked by significance of SNPs collapsed by gene and reported in Supplementary Tables 4.2 ("intermediate" class) and 4.4 ("declining" class). The number of SNPs tested in each gene was modestly correlated with the minimum p-value in that gene for "intermediate" (r = -0.42) and "declining" (r = -0.35) comparisons.

Figures 4.5 and 4.6 show p-values and linkage disequilibrium patterns for unpruned SNPs in *CNTN4* and *CNTNAP2* for the "intermediate" (panel A) and "declining" (panel B) class results and PGC ASD case-control results (panel C) ("Psychiatric Genomics Consortium,"). In *CNTN4,* the highest peaks, although different in location across latent class groups, reach a similar degree of significance to the PGC results for this gene. *CNTN4* also harbors variants associated with schizophrenia, intelligence, and brain connectivity. In *CNTNAP2*, the "declining" class exhibits a suggestive locus near known variants for bipolar disorder and schizophrenia, as well as late onset Alzheimer's disease (Hirano et al., 2015; K. S. Wang, Liu, & Aragam, 2010).

Suggestive PGC results in this gene map to the same location as a previously identified variant for Alzheimer's disease.

DISCUSSION

In this study, we sought to identify ASD-related genetic variants associated with differential trajectories of cognitive and motor development among infant high-risk ASD siblings. We used mixture modeling techniques to identify distinct trajectories of cognitive development among infant siblings of children with ASD who participated in the EARLI and IBIS cohort studies. We identified three subphenotypes of early cognitive and motor development: "normative", "intermediate", and "declining" classes. Subsequently, we tested for association of various ASD-related common genetic variants, including an ASD PRS and SNPs within 47 ASD candidate genes. Top ranked associated SNPs were largely in genes involved in neuronal migration during neurodevelopment and showed early trajectory effect sizes similar to those observed in ASD GWAS.

Three early developmental trajectory classes were identified. Approximately one third (33%) were classified into the "normative" class, where all domains exhibit a slight, steady increase in means from 6 to 36 months. Nearly half (46%) of the sample was classified into the "intermediate" class, where the pattern of MSEL mean domain-specific scores was similar to that of the "normative" class, but consistently lower by approximately 10 points (or 1 SD). The "declining" class included approximately 21% of the sample where mean MSEL scores exhibited a steady decline from 6 to 36 months. The "declining" class starts to differentiate from the "intermediate" class at 12 months for receptive language skills and 24 months for fine motor, visual reception, and expressive

language skills. The gross motor domain did not appear to differentiate well between classes. Previous work suggests that motor delays at 3 and 6 months precede later communication delays and other signs of ASD among high-risk ASD siblings compared to low-risk siblings (Bhat, Galloway, & Landa, 2012; Estes et al., 2015). We did not observe an obvious trend of motor deficits preceding communication deficits. However, one reason could be that we did not include low-risk (general population) siblings in our model; thus, we may not see an altogether higher functioning group that would otherwise be present. This is supported by the fact that our study means for MSEL domain scores are slightly lower (Table 4.1) than would be expected from a general population sample, as the MSEL domain t-scores are standardized at a population mean of 50 (Mullen, 1995). Additionally, Bhat et al. evaluated 6 month motor delay using a different instrument, which may be more sensitive to motor deficits than the MSEL (Bhat et al., 2012).

Approximately 22% of the full sample had a DSM-IV ASD diagnosis (Table 4.1). This is expected from previous reports of ASD risk among high-risk siblings (Ozonoff et al., 2011). Prevalence of ASD differed between groups, with ASD cases comprising approximately half (51.7%) of the "declining" class, 15% of the "intermediate" class, and 2% of the "normative" class. These results suggest that while cognitive developmental trajectory may be associated with ASD case status, it is not a direct surrogate for ASD, or ASD severity (Table 4.3). This is consistent with previous literature where ASD cases were spread among the lower scoring MSEL trajectory classes among high-risk ASD siblings (Landa et al., 2012).

ASD-defined polygenic risk scores using relatively stringent thresholds were not significantly associated with latent trajectory classes, although PRS based on liberal thresholds did associate with ASD and with intermediate class membership. There are several potential reasons for this finding. The ASD PRS is currently based on PGC autism GWAS findings that have very few statistically significant SNPs and wide confidence intervals. Therefore, PRS weights developed from those effect sizes may be imprecise. Further, the PRS was informed by ASD-associations, not developmental traits.

We considered specific candidate gene SNPs to examine whether genes with known ASD associations also show effects with specific developmental trajectories. Two SNPs reached suggestive significance in association with the "intermediate" class compared to the "normative" class. The top hit for the "intermediate" class was located in the *CNTN4* gene on chromosome 3. Gene-based results ranked *CNTN4* as the top gene for the intermediate class (Supplementary Table 4.2) and the second most significant gene for the "declining" class (Supplementary Table 4.4), though this may in part be due to the modest correlation we observed between the number of SNPs tested in a gene and the highest degree of significance reached for that gene. *CNTN4* encodes a member of the contactin family of immunoglobulins, which are cell adhesion molecules that mediate cell surface interactions during nervous system development (Shimoda & Watanabe, 2009). Gene mutations and deletions have been implicated in ASD in both candidate and genome-wide studies (Cottrell et al., 2011; Glessner et al., 2009; Guo et al., 2012; Roohi et al., 2009). *CNTN4* is also implicated in 3p deletion syndrome, which is associated with developmental delay (Dijkhuizen et al., 2006; Fernandez et al., 2008).

Regional mapping of unpruned SNPs in *CNTN4* revealed a peak of suggestive significance below rs4075530 (Figure 4.5). Although this SNP reaches only suggestive significance in our modest sample size (n=198), it is interesting to note that for this candidate gene, we see significance levels similar to that of the PGC with a much larger sample size of 10,610 (5,305 cases and 5,305 pseudocontrols) using ASD diagnostic status. This result argues for the utility of these trajectory phenotypes for gene discovery of both ASD and neurodevelopment generally; it could be true that the effect sizes for these phenotypes are larger and thus more detectible at lower sample sizes. Regardless, our result adds to existing evidence for the importance of *CNTN4* in neurodevelopment. There have been no other associations reported in this linkage disequilibrium block in the NHGRI GWAS catalog; however, several genome-wide significant associations for intelligence traits in a ADHD sample have been identified in the same gene nearby, approximately 50kb downstream (Loo et al., 2012).

The other SNP reaching suggestive significance was rs10265509 in the *CNTNAP2* gene on chromosome 7 for association with the "intermediate" class. This gene is highly expressed throughout the brain and spinal cord and encodes CASPR2, a protein known to have a role in regulating receptor-ligand interactions, cell adhesion, migration and differentiation (Rodenas-Cuadrado, Ho, & Vernes, 2014). In addition to autism (Arking et al., 2008), *CNTNAP2* has been implicated in many other neurodevelopmental and brain-related disorders, intellectual disability (Gregor et al., 2011), schizophrenia (Friedman et al., 2008), ADHD (Elia et al., 2010), and epilepsy (Mefford et al., 2010). Regional mapping of unpruned SNPs in *CNTNAP2* revealed an association peak with high linkage disequilibrium for "declining" class at the 5' end of the gene. This peak is

under previous findings implicating risk for bipolar disorder, schizophrenia (K. S. Wang et al., 2010), and Alzheimer's disease (Hirano et al., 2015). Therefore, our results contribute to the large body of evidence linking *CNTNAP2* to a broad range of neuropsychiatric phenotypes across the life course. This peak was less prominent in the "intermediate" class and the PGC case-control results.

We caution against over-interpretation of protective effects of many odds ratios reported among candidate gene SNPs. Our approach was to test SNPs that overlapped the genic boundaries of those reported in the SFARI database, so we did not limit testing to only previously associated SNPs, with defined risk alleles. We coded genotypes according to their dosage of the minor allele, as minor alleles confer risk more often than protection (Park et al., 2011). But in this enriched risk sample, it is less likely that the reference allele is the same as that in the general population. This potential for lack of concordance would be exacerbated at high MAFs, and we, indeed, used a relatively high MAF threshold (> 5%). Moreover, the propensity for minor alleles to confer risk is decreased at higher MAFs (Gorlova et al., 2012). Still, it could be true that minor alleles are less likely to be present in the "intermediate" or "declining" groups than the "normative" group. These SNPs could synonymous and/or located in intronic regions and, thus, would not have an effect on the candidate gene function. Future research with larger sample sizes should look to examine the consistency of these protective effects.

Limitations

This study is subject to several limitations. As with any longitudinal study, we had missing data across time points. This was due to an inability to attend an age-specific study visit and general loss to follow-up. Furthermore, EARLI ceased collection

of the MSEL at 24 month visits part way through the data collection phase due to funding constraints. However, we used a full information maximum likelihood (FIML) estimator in our PP-LCGA model, which is robust to missing data (Muthen & Shedden, 1999; Schafer & Graham, 2002). Second, the PRS was constructed from SNPs only modestly associated with ASD. Relating an ASD PRS to latent trajectory phenotypes should be attempted again once GWAS results from larger studies are available and genome-wide significant SNPs have been identified. Finally, our modest sample size precluded finding Bonferroni significant results in the candidate gene analyses. We plan to add genotype data for the IBIS portion of this sample in the near future, which will theoretically increase power.

Conclusion

Here, we report the first genetic association study with developmental trajectory classes among high-risk ASD siblings. We did not find an association between ASD PRS and latent trajectory classes for most PRS SNP association thresholds. However, latent class associations with SNPs in ASD candidate genes show similar magnitude of association to ASD-outcome results from much larger sample sizes, suggesting potential for further elucidation of mechanism when considering trajectory phenotypes compared to diagnostic categories. Genes coding for proteins involved in cell-cell interactions and neuronal migration in the developing nervous system may be specifically related to early cognitive developmental trajectory. Larger sample sizes are needed to replicate these modest findings. Future work should look beyond ASD candidate genes and focus on genome-wide pathway and gene-set analyses to further explore the genetic basis to differential trajectories of cognitive and motor development.

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Table 4.1. EARLI-IBIS collaborative cohort sample counts, descriptive statistics, and overall scores for Mullen Scales of Early Learning domains.

Study	**n**	**%**
EARLI	231	37.7
IBIS	382	62.3
DSM-IV ASD Diagnosis	109	22.4
Sex	**n**	**%**
Male	362	59.1
Female	251	40.9
Gross Motor	**mean**	**sd**
6 months	47.7	8.9
12 months	47.4	12.0
24 months	47.9	9.9
Fine Motor	**mean**	**sd**
6 months	48.8	9.6
12 months	56.2	9.5
24 months	47.9	10.7
36 months	44.4	13.8
Visual Reception	**mean**	**sd**
6 months	50.8	9.3
12 months	53.3	9.9
24 months	51.2	11.7
36 months	55.6	15.7
Receptive Language	**mean**	**sd**
6 months	50.0	9.6
12 months	42.7	9.1
24 months	48.1	13.5
36 months	46.7	11.2
Expressive Language	**mean**	**sd**
6 months	44.1	7.6
12 months	46.2	11.9
24 months	46.5	12.7
36 months	47.7	14.2

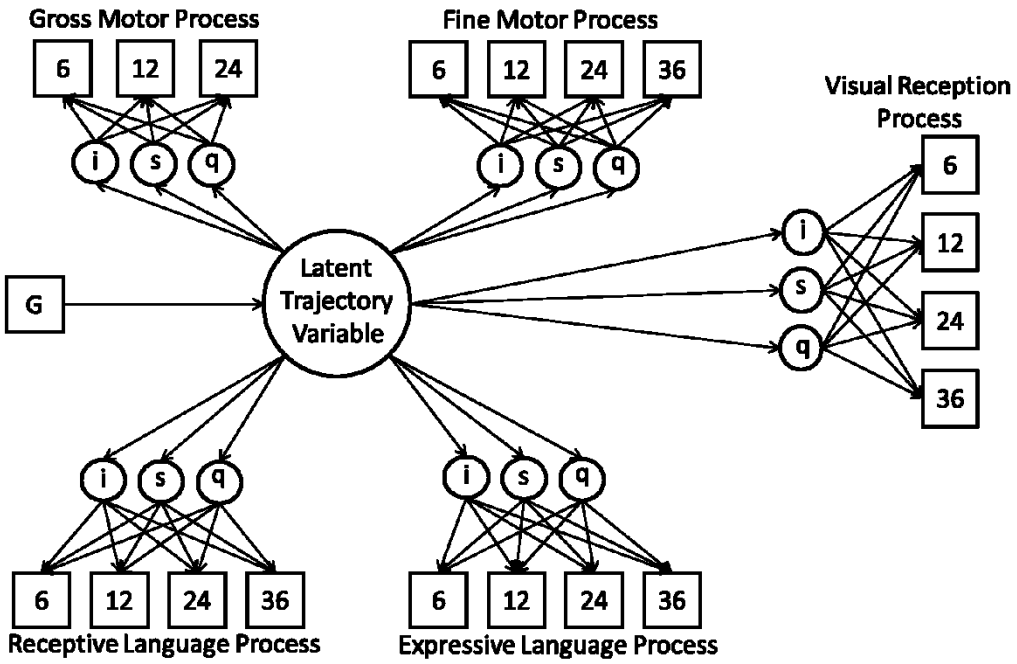


Figure 4.1. Parallel process LCGA model. Latent variable intercepts (i), slopes (s), and quadratic (q) terms are used to construct latent classes. The boxed G represents genetic predictors (ASD polygenic risk score, and ASD candidate gene SNPs) tested using latent class regression. Boxed numbers represent Mullen Scales of Early Learning domain-specific t-score measured at 6, 12, 24, and 36 month study visits.

Table 4.2. Fit statistics for 1- through 5-class LCGA models.

Classes	Free Parameters	Class proportions					Fit Statistics					
		Class 1	Class 2	Class 3	Class 4	Class 5	AIC[a]	BIC[b]	ssBIC[c]	VLMR[d]	BLRT[e]	Entropy
1	20	1	NA	NA	NA	NA	64796	64884	64821	NA	NA	NA
2	36	0.67	0.33	NA	NA	NA	63425	63584	63469	<0.001	<0.001	0.79
3	**52**	**0.46**	**0.21**	**0.33**	NA	NA	**63116**	**63345**	**63180**	**0.092**	**<0.001**	**0.73**
4	68	0.47	0.26	0.12	0.15	NA	62998	63299	63083	0.188	<0.001	0.74
5	84	0.24	0.11	0.26	0.25	0.14	62877	63248	62982	0.246	<0.001	0.70

[a]Akaike information criterion
[b]Bayesian information criterion
[c]Sample size adjusted Bayesian information criterion
[d]Vuong-Lo-Mendell-Rubin likelihood ratio test
[e]Bootstrap likelihood ratio test

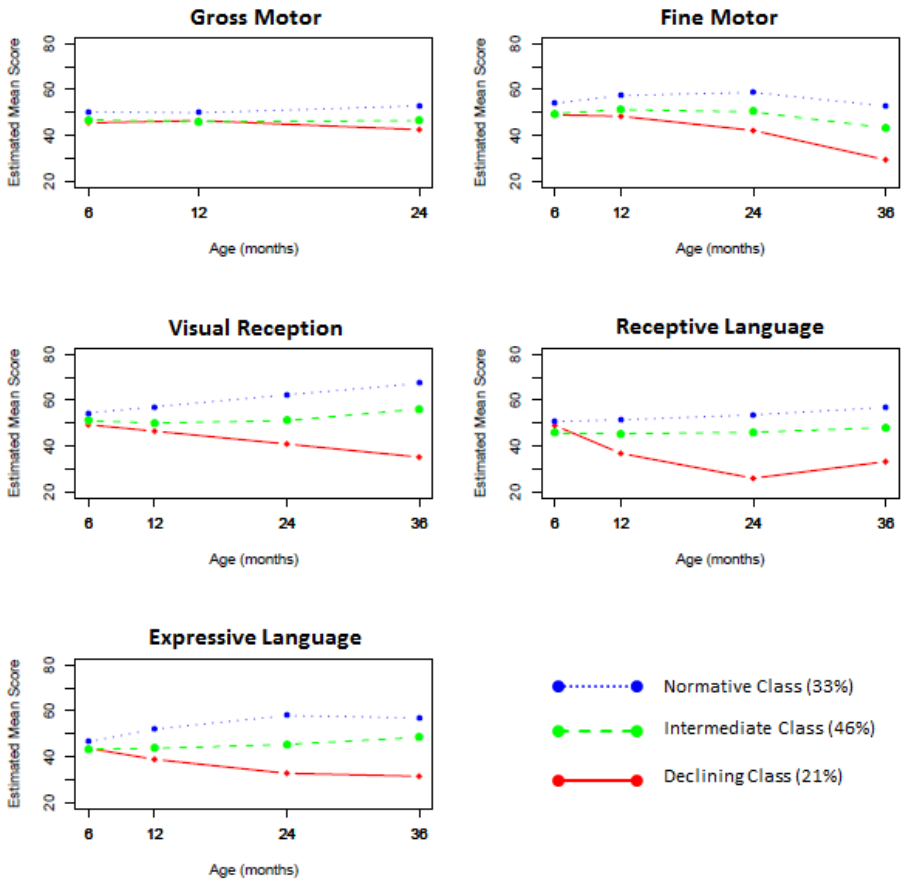


Figure 4.2. Estimated mean MSEL domain scores by class for 3-class LCGA solution. The classes are named "normative", "intermediate", and "declining" based on general patterns of MSEL subscale scores over time. Class proportions based on the estimated model are denoted in the figure legend.

Table 4.3. Distribution of autism spectrum disorder diagnostic status and sex by most-likely class assignment.

	Intermediate N=299		**Declining N=116**		**Normative N=198**	
	n	%	n	%	N	%
DSM-IV Diagnosis						
ASD [a]	46	15.4	60	51.7	3	1.5
Non-ASD [b]	185	61.9	39	33.6	154	77.8
Missing diagnosis	68	22.7	17	14.7	41	20.7
Sex						
Male	184	61.5	86	74.1	92	46.5
Female	115	38.5	30	25.9	106	53.5

[a] ASD = Autism spectrum disorders (autism, Asperger syndrome, or PDD-NOS)
[b] Non-ASD = the absence of an ASD after evaluation

Table 4.4. Standardized and quartiled PRS associations with class membership and ASD diagnosis.

	Standardized Score OR [a] (p)			***PRS Quartile 4 vs. PRS Quartile 1 OR [a] (p)***		
	PRS p-value threshold			**PRS p-value threshold**		
	1×10^{-3}	**1×10^{-4}**	**1×10^{-5}**	**1×10^{-3}**	**1×10^{-4}**	**1×10^{-5}**
Latent class						
Intermediate	1.06 (0.822)	1.25 (0.443)	0.92 (0.553)	0.68 (0.654)	1.38 (0.765)	0.31 (0.253)
Declining	1.66 (0.083)	1.12 (0.626)	0.99 (0.895)	1.98 (0.450)	1.76 (0.699)	0.50 (0.349)
Diagnosis						
ASD [b]	**1.64 (0.032)**	1.09 (0.706)	1.45 (0.096)	**7.72 (0.013)**	1.24 (0.744)	1.91 (0.328)

[a] Odds ratios are adjusted for sex and ancestral principal components 1 through 5.
[b] ASD = Autism spectrum disorders (autism, Asperger syndrome, or PDD-NOS)

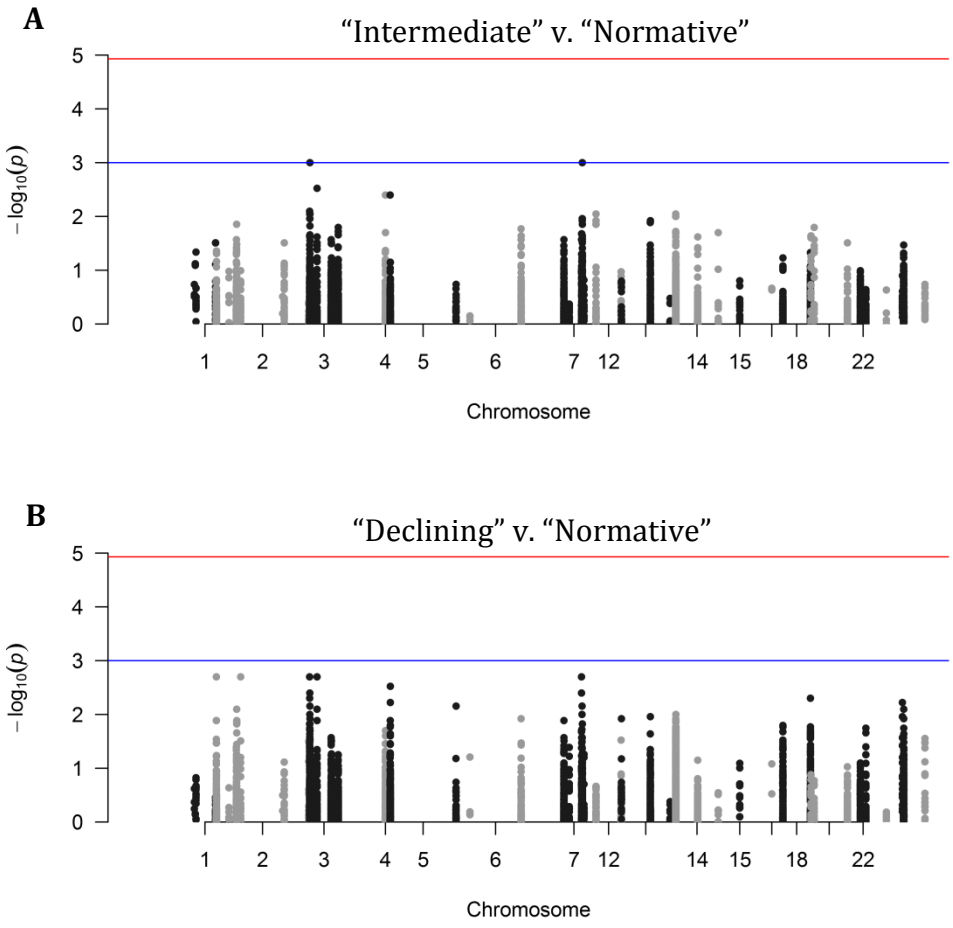


Figure 4.3. Manhattan plots of association p-values for 4,257 SNPs located in 47 candidate genes under an additive genetic model. P-values were obtained using a 3-step latent class multinomial logistic regression with the "normative" class as the reference category and adjusted for ancestry principal components 1 through 5 and gender. The horizontal blue line represents a threshold for suggestive significance defined at 1 x 10^{-3}. The horizontal red line represents a Bonferroni significant threshold at 1.2 x 10^{-5}. Panel s **A** and **B** show -$\log_{10}$ p-values by genomic position for the "intermediate" and "declining" class, respectively.

Table 4.5. Top 10 significant SNPs associated with the "intermediate" class relative to the "normative" class.

Chr.	Position	rsID	Gene	SFARI Gene Class	OR	*p*
3	2928197	NA	CNTN4	Strong Candidate	0.23	0.001
7	147815663	rs10265509	CNTNAP2	Strong Candidate	9.3	0.001
3	20173805	rs11705766	KAT2B	Strong Candidate	0.25	0.003
4	114001887	rs2135354	ANK2	High Confidence	0.14	0.004
5	11128927	rs67775011	CTNND2	Strong Candidate	0.15	0.004
3	2163838	NA	CNTN4	Strong Candidate	0.12	0.008
3	2425824	rs9830036	CNTN4	Strong Candidate	4.79	0.009
3	3014060	rs340804	CNTN4	Strong Candidate	0.21	0.009
10	28886626	rs332160	WAC	Strong Candidate	0.29	0.009
12	13753091	rs1805479	GRIN2B	High Confidence	0.15	0.009

Table 4.6. Top 10 significant SNPs associated with the "declining" class relative to the "normative" class.

Chr.	Position	rsID	Gene	SFARI Gene Class	OR	*p*
2	1859595	NA	MYT1L	Strong Candidate	4.01	0.002
2	60757467	rs202108972	BCL11A	Strong Candidate	0.27	0.002
3	2145996	rs2727926	CNTN4	Strong Candidate	0.32	0.002
3	20108644	rs1610186	KAT2B	Strong Candidate	4.68	0.002
7	145921523	rs201323315	CNTNAP2	Strong Candidate	4.07	0.002
5	11585335	rs2429307	CTNND2	Strong Candidate	0.29	0.003
3	2596520	rs79864813	CNTN4	Strong Candidate	4.53	0.004
7	145934145	rs17579058	CNTNAP2	Strong Candidate	0.31	0.004
3	2280429	NA	CNTN4	Strong Candidate	3.24	0.005
15	93501165	rs62023142	CHD2	Strong Candidate	3.5	0.005

Table 4.7. Top 10 jointly ranked SNPs associated with both the "intermediate" and "declining" classes.

CHR	Position	rsID	Gene	SFARI Gene Class	OR1[a]	*p*1[a]	OR2[b]	*p*2[b]
7	145934145	rs17579058	CNTNAP2	Strong Candidate	0.36	0.027	0.31	0.004
2	51201137	NA	NRXN1	Strong Candidate	0.35	0.033	0.46	0.014
15	93501165	rs62023142	CHD2	Strong Candidate	2.88	0.047	3.5	0.005
3	2928197	NA	CNTN4	Strong Candidate	0.23	0.001	0.39	0.029
12	13744039	rs34942586	GRIN2B	High Confidence	0.25	0.019	0.45	0.032
3	2425824	rs9830036	CNTN4	Strong Candidate	4.79	0.009	2.37	0.039
12	13740803	rs918092	GRIN2B	High Confidence	2.42	0.058	2.58	0.019
7	147980396	NA	CNTNAP2	Strong Candidate	0.42	0.069	4.41	0.015
12	13752832	rs2268097	GRIN2B	High Confidence	0.18	0.01	0.42	0.045
12	13753829	rs1805518	GRIN2B	High Confidence	0.18	0.01	0.42	0.045

[a]Intermediate class vs. normative class
[b]Declining class vs. normative class

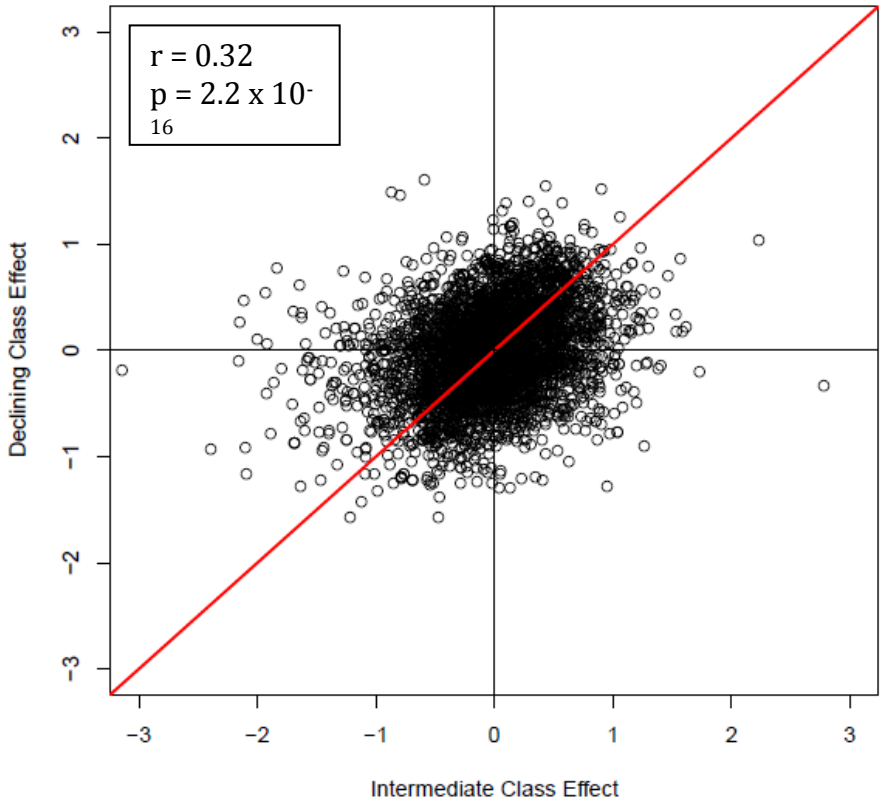


Figure 4.4. Correlation of effect sizes (log odds ratios) between the "intermediate" class and "declining" class for all candidate gene SNPs tested. Effect sizes were moderately correlated ($r=0.32$, $p = 2.2 \times 10^{16}$).

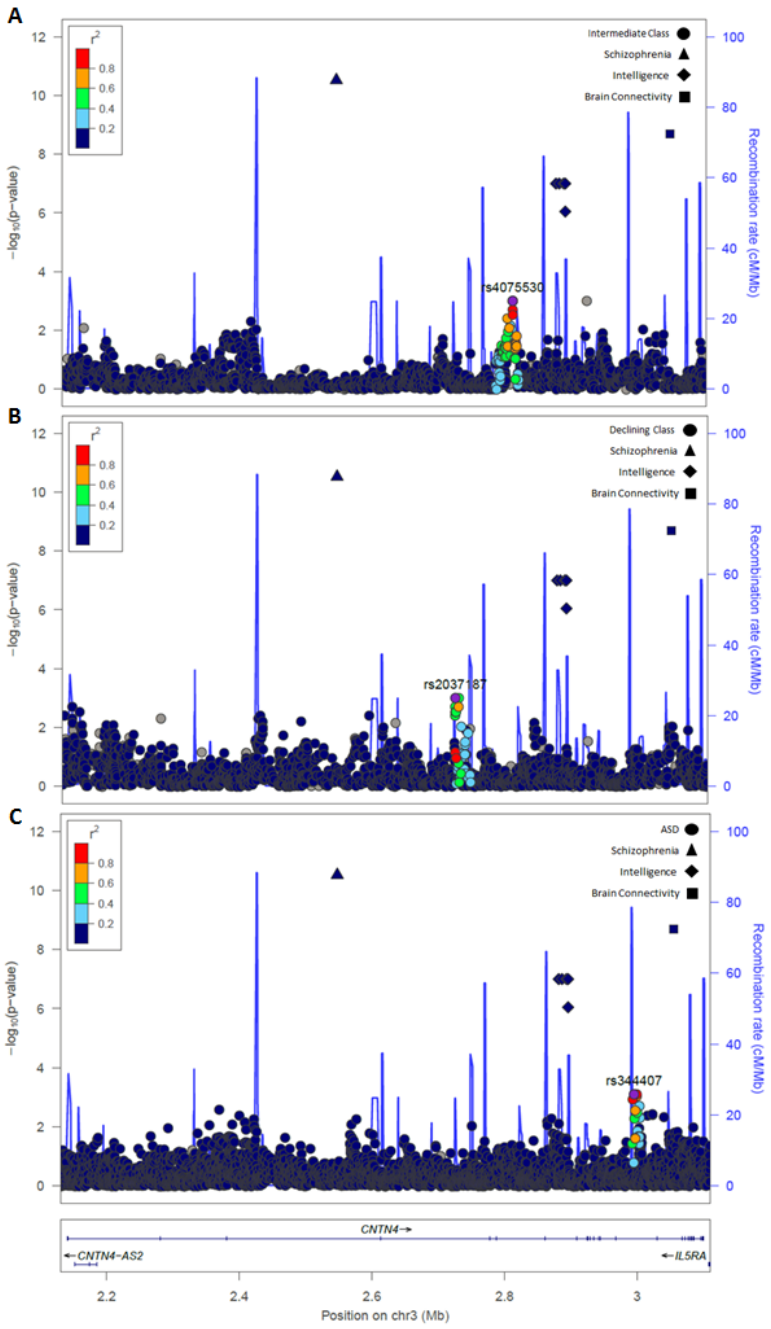


Figure 4.5. Regional plots for unpruned SNPs in *CNTN4*. Left y-axis indicates degree of significance ($-\log_{10}$p-value) for association tests of unpruned SNPs tested in *CNTN4*. Right y-axis depicts recombination rates. X-axis depicts genomic position. Points are shaped in accordance with whether they were tested for association in EARLI (for panels A and B) (n=198) or the ASD PGC study (Panel C) (n=10,610) or are SNPs that have been previously demonstrated to be associated with another neuropsychiatric phenotype. Points are colored according the extent to which that SNP is in linkage disequilibrium (via r^2) with the most statistically significant SNP in the gene. **A**) Results for association testing comparing "intermediate" class to "normative" class. **B**) Results for association testing comparing "declining" class to normative class. **C**) ASD case-control PGC results.

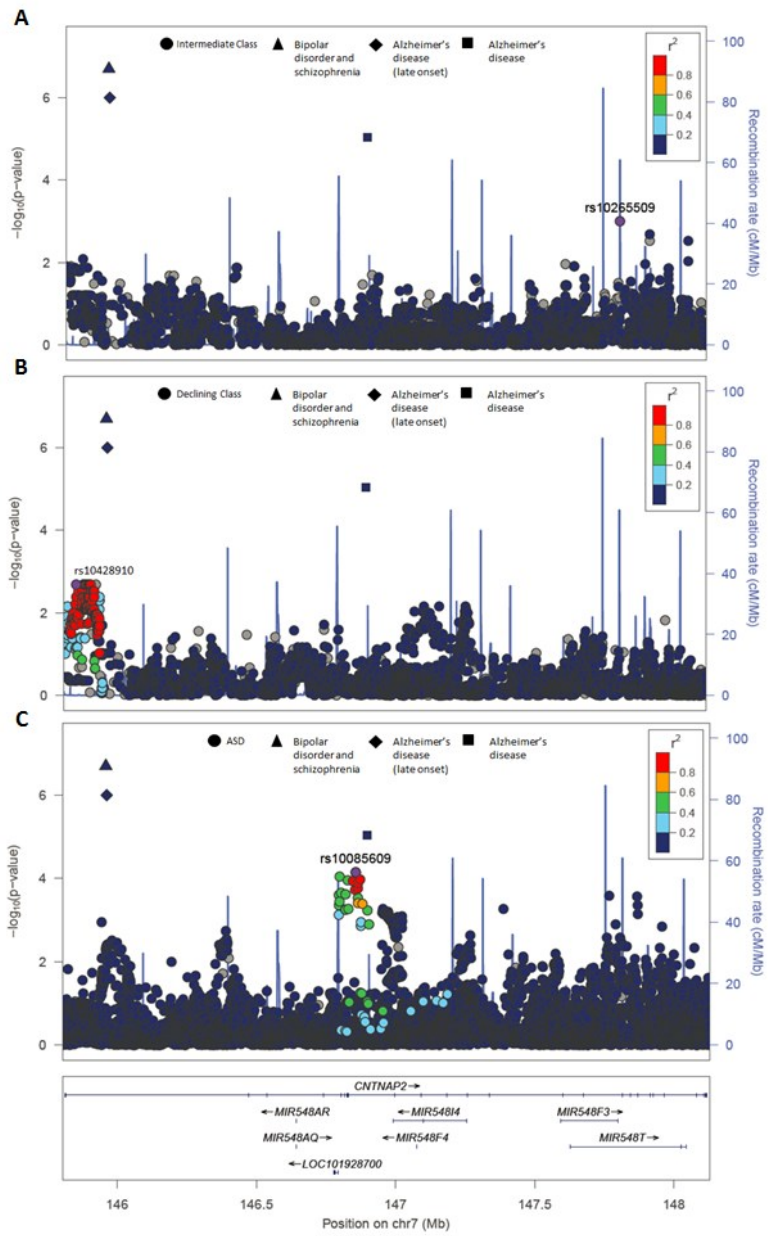
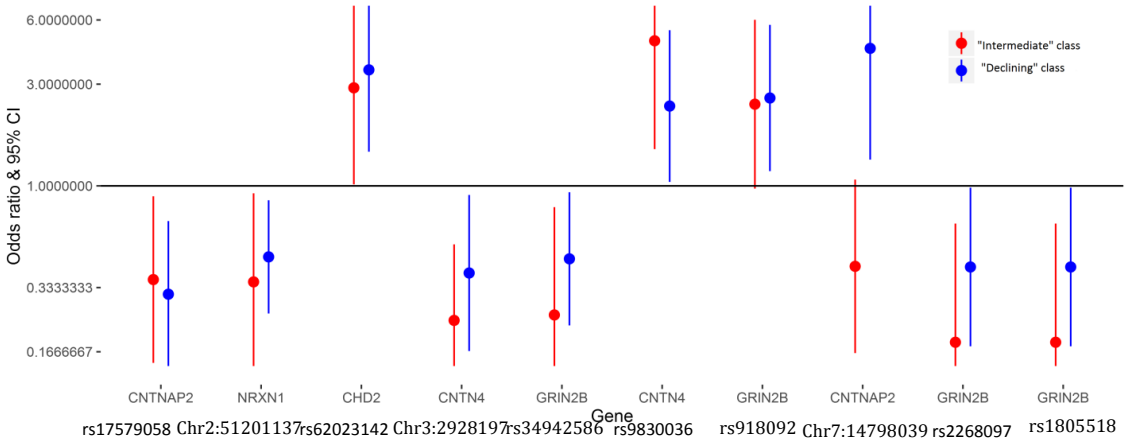


Figure 4.6. Regional plots for unpruned SNPs in *CNTNAP2*. Left y-axis indicates degree of significance (-$\log_{10}$p-value) for association tests of unpruned SNPs tested in *CNTNAP2*. Right y-axis depicts recombination rates. X-axis depicts genomic position. Points are shaped in accordance with whether they were tested for association in EARLI (for panels A and B) (n=198) or the ASD PGC study (n=10,610) (Panel C) or are SNPs that have been previously demonstrated to be associated with another neuropsychiatric phenotype. Points are colored according the extent to which that SNP is in linkage disequilibrium (via r^2) with the most statistically significant SNP in the gene. **A**) Results for association testing comparing "intermediate" class to "normative" class. **B**) Results for association testing comparing "declining" class to normative class. **C**) ASD case-control PGC results.



Supplementary Figure 4.1. Odds ratios and 95% confidence intervals for the top 10 jointly ranked SNPs in candidate genes tested under an additive model. ORs and CIs were obtained using a 3-step latent class multinomial logistic regression with the "normative" class as the reference category and adjusted for ancestry principal components 1 through 5 and gender. Red and blue represent ORs and CIs for the "intermediate" and "declining" class, respectively.

Supplementary Table 4.1. 117 Nominally significant ($p < 0.05$) SNPs associated with the "intermediate" class compared to the "normative" class.

Chr.	Position	rsID	Gene	SFARI Gene Class	OR	*p*
3	2928197	NA	CNTN4	Strong Candidate	0.23	0.001
7	147815663	rs10265509	CNTNAP2	Strong Candidate	9.3	0.001
3	20173805	rs11705766	KAT2B	Strong Candidate	0.25	0.003
4	114001887	rs2135354	ANK2	High Confidence	0.14	0.004
5	11128927	rs67775011	CTNND2	Strong Candidate	0.15	0.004
3	2163838	NA	CNTN4	Strong Candidate	0.12	0.008
3	2425824	rs9830036	CNTN4	Strong Candidate	4.79	0.009
3	3014060	rs340804	CNTN4	Strong Candidate	0.21	0.009
10	28886626	rs332160	WAC	Strong Candidate	0.29	0.009
12	13753091	rs1805479	GRIN2B	High Confidence	0.15	0.009
12	13752832	rs2268097	GRIN2B	High Confidence	0.18	0.01
12	13753829	rs1805518	GRIN2B	High Confidence	0.18	0.01
12	13753852	rs1805519	GRIN2B	High Confidence	0.18	0.01
3	2420429	rs9826250	CNTN4	Strong Candidate	0.26	0.011
3	2811719	rs60667714	CNTN4	Strong Candidate	5.64	0.011
7	147617447	NA	CNTNAP2	Strong Candidate	3.45	0.011
7	147905708	rs11975702	CNTNAP2	Strong Candidate	0.19	0.011
7	147649402	rs79028592	CNTNAP2	Strong Candidate	3.32	0.012
10	28841769	rs332122	WAC	Strong Candidate	0.26	0.012
10	28869729	rs332147	WAC	Strong Candidate	0.26	0.012
11	70813928	rs11823567	SHANK2	Strong Candidate	0.26	0.012
11	70421468	rs11236691	SHANK2	Strong Candidate	0.18	0.013
2	51003771	NA	NRXN1	Strong Candidate	0.29	0.014
7	147991580	rs10155900	CNTNAP2	Strong Candidate	0.29	0.014
10	28832820	rs61848477	WAC	Strong Candidate	0.3	0.014
10	28843854	rs332125	WAC	Strong Candidate	0.3	0.014
10	28862152	rs332141	WAC	Strong Candidate	0.3	0.014
3	2926235	rs17646423	CNTN4	Strong Candidate	0.25	0.015
3	71462441	rs73119151	FOXP1	Strong Candidate	0.32	0.016
16	9036281	rs2447931	USP7	Strong Candidate	0.16	0.016
6	157213008	NA	ARID1B	High Confidence	3.39	0.017
3	71374638	rs201114438	FOXP1	Strong Candidate	0.09	0.019
12	13744039	rs34942586	GRIN2B	High Confidence	0.25	0.019
4	114167779	rs362497	ANK2	High Confidence	3.17	0.02
12	13743245	rs2041889	GRIN2B	High Confidence	0.28	0.02
12	13812566	rs35222999	GRIN2B	High Confidence	3.87	0.02
12	13957091	rs202127623	GRIN2B	High Confidence	3.66	0.02
12	116546195	NA	MED13L	Strong Candidate	2.85	0.02

7	146179491	NA	CNTNAP2	Strong Candidate	3.67	0.021
7	146194998	NA	CNTNAP2	Strong Candidate	4.33	0.021
7	146422410	rs344461	CNTNAP2	Strong Candidate	4.88	0.021
12	13804359	rs2284404	GRIN2B	High Confidence	0.2	0.021
3	2884787	rs67182448	CNTN4	Strong Candidate	0.46	0.022
3	71434958	rs13070452	FOXP1	Strong Candidate	0.2	0.022
3	2198047	rs57594411	CNTN4	Strong Candidate	0.44	0.023
6	157432660	rs4869907	ARID1B	High Confidence	0.31	0.023
7	147923566	rs12532590	CNTNAP2	Strong Candidate	0.35	0.023
16	1249494	rs12918213	CACNA1H	Strong Candidate	2.36	0.023
16	1249495	rs12922159	CACNA1H	Strong Candidate	2.36	0.023
3	2413302	rs6795931	CNTN4	Strong Candidate	0.38	0.024
3	20162216	rs7617840	KAT2B	Strong Candidate	0.36	0.024
12	13739350	rs10772694	GRIN2B	High Confidence	0.23	0.024
12	66994814	rs7131878	GRIP1	Strong Candidate	0.4	0.024
16	1236190	rs58094843	CACNA1H	Strong Candidate	0.33	0.025
7	147960601	rs12704004	CNTNAP2	Strong Candidate	0.37	0.026
2	50476423	rs1712914	NRXN1	Strong Candidate	2.41	0.027
3	54327590	NA	CACNA2D3	Strong Candidate	0.4	0.027
6	157266505	rs4870499	ARID1B	High Confidence	2.6	0.027
7	103400437	NA	RELN	Strong Candidate	0.3	0.027
7	145934145	rs17579058	CNTNAP2	Strong Candidate	0.36	0.027
16	8995927	rs2304466	USP7	Strong Candidate	2.95	0.027
3	3043267	rs163353	CNTN4	Strong Candidate	0.41	0.028
7	147854500	rs10256938	CNTNAP2	Strong Candidate	0.35	0.029
12	14079894	rs200556225	GRIN2B	High Confidence	0.36	0.029
1	202695165	rs4950740	KDM5B	Strong Candidate	0.27	0.031
1	202718588	rs7519619	KDM5B	Strong Candidate	0.27	0.031
1	202730535	rs7551390	KDM5B	Strong Candidate	0.27	0.031
2	166238034	rs7581427	SCN2A	High Confidence	0.31	0.031
3	20112234	rs73179773	KAT2B	Strong Candidate	4.62	0.031
3	54896405	NA	CACNA2D3	Strong Candidate	2.44	0.031
7	147808629	NA	CNTNAP2	Strong Candidate	0.34	0.031
16	89507995	rs2911268	ANKRD11	Strong Candidate	3.02	0.031
3	54660737	rs74500931	CACNA2D3	Strong Candidate	2.93	0.032
3	54958762	rs201096330	CACNA2D3	Strong Candidate	0.2	0.032
2	51201137	NA	NRXN1	Strong Candidate	0.35	0.033
12	13805633	rs10845819	GRIN2B	High Confidence	0.2	0.033
3	2195692	rs55883839	CNTN4	Strong Candidate	0.24	0.034
11	70418346	rs10899191	SHANK2	Strong Candidate	3.01	0.034
21	41898497	rs8131731	DSCAM	Strong Candidate	2.71	0.034

3	20117417	rs76536306	KAT2B	Strong Candidate	2.43	0.035
6	157250189	rs284430	ARID1B	High Confidence	3.46	0.035
6	157404463	rs12197388	ARID1B	High Confidence	2.48	0.035
7	103153373	rs3808036	RELN	Strong Candidate	5.03	0.035
12	13762597	rs2268098	GRIN2B	High Confidence	0.29	0.035
12	13763455	rs1805481	GRIN2B	High Confidence	0.29	0.035
16	9035781	rs56410892	USP7	Strong Candidate	0.17	0.035
3	71164888	NA	FOXP1	Strong Candidate	3.05	0.037
3	71210210	rs34347778	FOXP1	Strong Candidate	0.35	0.037
6	157227472	rs479079	ARID1B	High Confidence	0.28	0.037
2	50600432	rs3081368	NRXN1	Strong Candidate	0.37	0.038
3	71065947	rs3846030	FOXP1	Strong Candidate	3	0.038
12	66982974	rs76650031	GRIP1	Strong Candidate	0.34	0.038
7	147993238	rs4726946	CNTNAP2	Strong Candidate	2.4	0.039
12	67028845	rs7311299	GRIP1	Strong Candidate	0.4	0.04
16	8996636	rs4985062	USP7	Strong Candidate	3.29	0.04
3	2391613	rs1720194	CNTN4	Strong Candidate	3.39	0.041
3	20179892	rs201896750	KAT2B	Strong Candidate	2.62	0.041
11	70470633	rs4980629	SHANK2	Strong Candidate	0.42	0.041
2	50817470	rs6733430	NRXN1	Strong Candidate	0.37	0.042
4	114005198	rs7676179	ANK2	High Confidence	3.25	0.043
7	103417134	rs262338	RELN	Strong Candidate	0.45	0.043
2	2223972	rs11127372	MYT1L	Strong Candidate	0.32	0.044
7	103397953	rs1476446	RELN	Strong Candidate	0.38	0.044
2	2289761	NA	MYT1L	Strong Candidate	3.31	0.045
1	155398245	rs9787192	ASH1L	High Confidence	2.69	0.046
2	50334395	NA	NRXN1	Strong Candidate	2.75	0.047
2	50804847	rs113038770	NRXN1	Strong Candidate	2.39	0.047
4	113745292	NA	ANK2	High Confidence	0.43	0.047
15	93501165	rs62023142	CHD2	Strong Candidate	2.88	0.047
16	9035124	rs1471435	USP7	Strong Candidate	2.32	0.047
2	2240556	rs150627539	MYT1L	Strong Candidate	2.27	0.048
11	70492808	rs11237057	SHANK2	Strong Candidate	3.58	0.048
21	41800106	rs2837623	DSCAM	Strong Candidate	2.45	0.048
2	2288632	rs4998343	MYT1L	Strong Candidate	0.16	0.049
3	2819988	rs112758727	CNTN4	Strong Candidate	2.46	0.049
7	103259560	rs201553109	RELN	Strong Candidate	2.35	0.049
7	2928197	NA	CNTNAP2	Strong Candidate	0.29	0.049

Supplementary Table 4.2. All candidate genes ranked by association with the "intermediate" class compared the "normative" class, based on SNP-level results collapsed by gene.

CHR	Gene	SFARI Gene Class	SNP Count	*p*
3	CNTN4	Strong Candidate	432	0.001
7	CNTNAP2	Strong Candidate	446	0.001
3	KAT2B	Strong Candidate	48	0.003
4	ANK2	High Confidence	130	0.004
5	CTNND2	Strong Candidate	188	0.004
10	WAC	Strong Candidate	92	0.009
12	GRIN2B	High Confidence	584	0.009
11	SHANK2	Strong Candidate	146	0.012
2	NRXN1	Strong Candidate	177	0.014
3	FOXP1	Strong Candidate	150	0.016
16	USP7	Strong Candidate	15	0.016
6	ARID1B	High Confidence	64	0.017
12	MED13L	Strong Candidate	9	0.02
16	CACNA1H	Strong Candidate	16	0.023
12	GRIP1	Strong Candidate	213	0.024
3	CACNA2D3	Strong Candidate	244	0.027
7	RELN	Strong Candidate	147	0.027
1	KDM5B	Strong Candidate	39	0.031
2	SCN2A	High Confidence	27	0.031
16	ANKRD11	Strong Candidate	59	0.031
21	DSCAM	Strong Candidate	255	0.034
2	MYT1L	Strong Candidate	98	0.044
1	ASH1L	High Confidence	20	0.046
15	CHD2	Strong Candidate	95	0.047
15	GABRB3	Strong Candidate	162	0.059
1	ILF2	Strong Candidate	4	0.076
2	BCL11A	Strong Candidate	20	0.102
18	ASXL3	High Confidence	99	0.102
3	SETD5	High Confidence	12	0.103
3	SLC6A1	Strong Candidate	21	0.103
2	SPAST	Strong Candidate	8	0.105
10	PTEN	High Confidence	24	0.107
21	DYRK1A	High Confidence	16	0.126
7	KMT2C	Strong Candidate	15	0.152
13	INTS6	Strong Candidate	13	0.157
11	DEAF1	Strong Candidate	25	0.159
5	RANBP17	Strong Candidate	23	0.183

22	SHANK3	High Confidence	17	0.183
1	POGZ	High Confidence	3	0.184
14	IRF2BPL	Strong Candidate	2	0.216
18	KATNAL2	Strong Candidate	53	0.229
20	ADNP	High Confidence	4	0.233
2	TBR1	High Confidence	2	0.309
11	KMT2A	Strong Candidate	8	0.334
2	CUL3	Strong Candidate	11	0.393
7	MET	Strong Candidate	18	0.426
6	SYNGAP1	High Confidence	3	0.707

Supplementary Table 4.3. 251 Nominally significant ($p < 0.05$) SNPs associated with the "declining" class compared to the "normative" class.

CHR	Position	rsID	Gene	SFARI Gene Class	OR	*p*
2	1859595	NA	MYT1L	Strong Candidate	4.01	0.002
2	60757467	rs202108972	BCL11A	Strong Candidate	0.27	0.002
3	2145996	rs2727926	CNTN4	Strong Candidate	0.32	0.002
3	20108644	rs1610186	KAT2B	Strong Candidate	4.68	0.002
7	145921523	rs201323315	CNTNAP2	Strong Candidate	4.07	0.002
5	11585335	rs2429307	CTNND2	Strong Candidate	0.29	0.003
3	2596520	rs79864813	CNTN4	Strong Candidate	4.53	0.004
7	145934145	rs17579058	CNTNAP2	Strong Candidate	0.31	0.004
3	2280429	NA	CNTN4	Strong Candidate	3.24	0.005
15	93501165	rs62023142	CHD2	Strong Candidate	3.5	0.005
5	11555821	NA	CTNND2	Strong Candidate	0.39	0.006
21	38744586	rs2409894	DYRK1A	High Confidence	0.38	0.006
3	2846838	rs12633320	CNTN4	Strong Candidate	3.34	0.007
5	170704942	NA	RANBP17	Strong Candidate	2.65	0.007
7	147258631	rs6960319	CNTNAP2	Strong Candidate	2.82	0.007
2	51089340	NA	NRXN1	Strong Candidate	3.97	0.008
3	20103476	NA	KAT2B	Strong Candidate	4.95	0.008
21	41997529	rs9974484	DSCAM	Strong Candidate	2.52	0.008
3	2617954	rs2728056	CNTN4	Strong Candidate	0.33	0.01
7	147121340	rs7794718	CNTNAP2	Strong Candidate	0.33	0.01
12	14024769	rs10845851	GRIN2B	High Confidence	0.21	0.01
11	70692076	rs7941869	SHANK2	Strong Candidate	0.29	0.011
21	38817390	rs9982990	DYRK1A	High Confidence	0.4	0.011
3	3080200	rs7617493	CNTN4	Strong Candidate	0.42	0.012
6	157216039	rs184074	ARID1B	High Confidence	2.63	0.012
11	686127	NA	DEAF1	Strong Candidate	2.45	0.012
12	13803051	rs2300234	GRIN2B	High Confidence	2.53	0.012
21	42024988	rs78896678	DSCAM	Strong Candidate	0.3	0.012
2	1858603	rs6709806	MYT1L	Strong Candidate	3.59	0.013
2	1874731	rs11675862	MYT1L	Strong Candidate	0.25	0.013
2	51257248	NA	NRXN1	Strong Candidate	0.43	0.013
3	20183278	rs75731241	KAT2B	Strong Candidate	0.34	0.013
5	11354932	rs1363940	CTNND2	Strong Candidate	2.63	0.013
7	103411103	NA	RELN	Strong Candidate	2.34	0.013
12	13948309	NA	GRIN2B	High Confidence	0.39	0.013
2	51201137	NA	NRXN1	Strong Candidate	0.46	0.014
12	13813330	rs2300238	GRIN2B	High Confidence	2.53	0.014
12	14087122	rs7295850	GRIN2B	High Confidence	2.57	0.014

2	51257062	rs34300360	NRXN1	Strong Candidate	0.31	0.015
3	2187631	NA	CNTN4	Strong Candidate	0.29	0.015
3	2206373	rs2727927	CNTN4	Strong Candidate	0.36	0.015
3	2209756	rs2727930	CNTN4	Strong Candidate	0.29	0.015
3	2746456	rs13080275	CNTN4	Strong Candidate	3	0.015
7	147980396	NA	CNTNAP2	Strong Candidate	4.41	0.015
5	11538818	rs60883018	CTNND2	Strong Candidate	0.41	0.016
15	26876249	rs751994	GABRB3	Strong Candidate	2.83	0.016
5	11316583	rs113400641	CTNND2	Strong Candidate	2.45	0.017
12	13939697	rs78812890	GRIN2B	High Confidence	3.32	0.017
12	14068789	rs6488624	GRIN2B	High Confidence	2.22	0.017
12	14070156	rs2216344	GRIN2B	High Confidence	2.22	0.017
12	14083859	rs219885	GRIN2B	High Confidence	0.26	0.017
12	14084808	rs219887	GRIN2B	High Confidence	0.29	0.017
12	14085176	rs219888	GRIN2B	High Confidence	0.29	0.017
12	14091037	rs219902	GRIN2B	High Confidence	0.29	0.017
15	26981846	rs7179279	GABRB3	Strong Candidate	0.33	0.017
15	93520108	rs34026217	CHD2	Strong Candidate	0.33	0.017
3	2465934	rs76882636	CNTN4	Strong Candidate	2.78	0.018
12	13950074	rs11055623	GRIN2B	High Confidence	2.91	0.018
12	13950577	rs7974275	GRIN2B	High Confidence	2.91	0.018
12	14075430	rs219872	GRIN2B	High Confidence	0.3	0.018
12	14076446	rs219873	GRIN2B	High Confidence	0.3	0.018
12	14085886	NA	GRIN2B	High Confidence	2.33	0.018
12	14085920	rs28695969	GRIN2B	High Confidence	2.33	0.018
12	14087599	rs219892	GRIN2B	High Confidence	0.3	0.018
18	44557886	rs138970065	KATNAL2	Strong Candidate	2.6	0.018
21	42026025	NA	DSCAM	Strong Candidate	0.29	0.018
3	2194392	rs2729017	CNTN4	Strong Candidate	0.24	0.019
3	2588293	rs35787589	CNTN4	Strong Candidate	3.13	0.019
3	2616751	rs734246	CNTN4	Strong Candidate	0.4	0.019
7	146747059	rs11983645	CNTNAP2	Strong Candidate	2.26	0.019
12	13740803	rs918092	GRIN2B	High Confidence	2.58	0.019
15	93523632	rs2033844	CHD2	Strong Candidate	0.34	0.019
3	3057903	rs3907591	CNTN4	Strong Candidate	0.3	0.02
4	114154261	NA	ANK2	High Confidence	0.41	0.02
7	145943555	rs148812117	CNTNAP2	Strong Candidate	2.55	0.02
12	14082733	rs219879	GRIN2B	High Confidence	0.31	0.02
12	14083520	rs219882	GRIN2B	High Confidence	0.31	0.02
7	145831671	rs62504792	CNTNAP2	Strong Candidate	0.38	0.021
12	13890358	rs2110983	GRIN2B	High Confidence	0.38	0.021

12	13948580	rs220561	GRIN2B	High Confidence	0.43	0.021
12	14088861	rs219894	GRIN2B	High Confidence	0.28	0.021
12	14092888	rs219904	GRIN2B	High Confidence	2.2	0.021
15	26834590	rs11161319	GABRB3	Strong Candidate	2.7	0.021
15	93498105	rs3817597	CHD2	Strong Candidate	0.35	0.021
15	93537958	rs17525800	CHD2	Strong Candidate	0.34	0.021
2	51186984	rs10197386	NRXN1	Strong Candidate	0.35	0.022
12	13734420	rs12308472	GRIN2B	High Confidence	2.33	0.022
12	13734426	NA	GRIN2B	High Confidence	2.33	0.022
12	13912776	rs7296762	GRIN2B	High Confidence	2.38	0.022
12	13954403	rs220567	GRIN2B	High Confidence	2.4	0.022
15	93536804	rs62023149	CHD2	Strong Candidate	3.11	0.022
15	93536811	rs58966536	CHD2	Strong Candidate	3.01	0.022
18	44556883	NA	KATNAL2	Strong Candidate	0.37	0.022
3	2575184	NA	CNTN4	Strong Candidate	0.39	0.023
5	11520099	NA	CTNND2	Strong Candidate	3.4	0.023
11	70488398	rs7946437	SHANK2	Strong Candidate	2.28	0.023
12	13959245	rs220581	GRIN2B	High Confidence	2.44	0.023
12	14083631	rs219884	GRIN2B	High Confidence	2.19	0.023
12	14090624	rs219900	GRIN2B	High Confidence	2.19	0.023
15	93526003	rs11634207	CHD2	Strong Candidate	0.36	0.023
21	42130162	rs734571	DSCAM	Strong Candidate	0.42	0.023
12	13953118	rs2058878	GRIN2B	High Confidence	2.49	0.024
15	93473624	rs961233	CHD2	Strong Candidate	0.4	0.024
4	114155056	rs362462	ANK2	High Confidence	0.31	0.025
5	11578574	rs7737639	CTNND2	Strong Candidate	0.43	0.025
12	13749831	rs1805508	GRIN2B	High Confidence	2.49	0.025
12	14096444	rs219915	GRIN2B	High Confidence	2.17	0.025
15	93540089	rs11630582	CHD2	Strong Candidate	0.37	0.025
3	2576262	rs35501813	CNTN4	Strong Candidate	2.91	0.026
12	13901815	rs1005549	GRIN2B	High Confidence	3.71	0.026
12	14004787	rs2193511	GRIN2B	High Confidence	2.37	0.026
12	14095457	rs11055693	GRIN2B	High Confidence	0.31	0.026
15	93531312	NA	CHD2	Strong Candidate	0.36	0.026
15	93546474	rs12906132	CHD2	Strong Candidate	0.35	0.026
3	54280586	NA	CACNA2D3	Strong Candidate	2.6	0.027
7	103397082	rs3819479	RELN	Strong Candidate	2.6	0.027
7	146291525	rs368392827	CNTNAP2	Strong Candidate	0.42	0.027
7	147692624	rs5003321	CNTNAP2	Strong Candidate	2.15	0.027
12	13734261	rs1806197	GRIN2B	High Confidence	2.09	0.027
12	13956579	rs2300272	GRIN2B	High Confidence	2.39	0.027

15	93473251	rs201925722	CHD2	Strong Candidate	0.41	0.027
15	93473752	rs1318689	CHD2	Strong Candidate	0.41	0.027
21	38817699	rs9974713	DYRK1A	High Confidence	0.3	0.027
21	41631588	rs372505754	DSCAM	Strong Candidate	0.4	0.027
12	13975298	rs220599	GRIN2B	High Confidence	0.42	0.028
12	13975719	NA	GRIN2B	High Confidence	0.42	0.028
12	14109687	rs219931	GRIN2B	High Confidence	0.33	0.028
12	14117136	rs219936	GRIN2B	High Confidence	0.37	0.028
15	93464509	rs28573213	CHD2	Strong Candidate	0.42	0.028
22	51146740	rs9628239	SHANK3	High Confidence	0.46	0.028
2	1865275	NA	MYT1L	Strong Candidate	0.35	0.029
3	2423855	NA	CNTN4	Strong Candidate	0.28	0.029
3	2581603	rs9878895	CNTN4	Strong Candidate	2.62	0.029
3	2636695	rs2616597	CNTN4	Strong Candidate	2.23	0.029
3	2928197	NA	CNTN4	Strong Candidate	0.39	0.029
3	54297185	rs7614823	CACNA2D3	Strong Candidate	2.9	0.029
12	13956734	rs220573	GRIN2B	High Confidence	0.43	0.029
12	13957286	rs220575	GRIN2B	High Confidence	0.43	0.029
12	14082188	rs219878	GRIN2B	High Confidence	2.14	0.029
10	89626375	NA	PTEN	High Confidence	0.41	0.03
12	13886268	rs10492135	GRIN2B	High Confidence	3.21	0.03
12	13886646	rs11055606	GRIN2B	High Confidence	3.21	0.03
12	13887243	rs73051594	GRIN2B	High Confidence	3.21	0.03
12	13893865	rs17833849	GRIN2B	High Confidence	3.21	0.03
12	13898935	rs73053620	GRIN2B	High Confidence	3.21	0.03
12	13898968	rs77252419	GRIN2B	High Confidence	3.21	0.03
12	13899012	NA	GRIN2B	High Confidence	3.21	0.03
15	26838405	rs12437672	GABRB3	Strong Candidate	2.53	0.03
2	60777498	rs6747099	BCL11A	Strong Candidate	0.46	0.031
3	54894545	rs1526596	CACNA2D3	Strong Candidate	0.45	0.031
7	103624813	rs12667675	RELN	Strong Candidate	2.58	0.031
12	14007590	NA	GRIN2B	High Confidence	0.44	0.031
12	14093981	rs219906	GRIN2B	High Confidence	2.12	0.031
2	225399718	NA	CUL3	Strong Candidate	2.18	0.032
3	2725785	rs1878181	CNTN4	Strong Candidate	2.12	0.032
7	147244724	rs2190008	CNTNAP2	Strong Candidate	0.44	0.032
12	13744039	rs34942586	GRIN2B	High Confidence	0.45	0.032
3	54308840	rs11130410	CACNA2D3	Strong Candidate	2.3	0.033
12	13734262	rs2058771	GRIN2B	High Confidence	2.05	0.033
12	13967591	rs220595	GRIN2B	High Confidence	0.39	0.033
12	14078634	rs918168	GRIN2B	High Confidence	2.53	0.033

12	14089032	rs1421104	GRIN2B	High Confidence	0.28	0.033
15	26852038	rs12904322	GABRB3	Strong Candidate	2.52	0.033
21	42162117	rs28663497	DSCAM	Strong Candidate	0.36	0.033
22	51126128	rs9616937	SHANK3	High Confidence	0.47	0.033
2	1890939	NA	MYT1L	Strong Candidate	0.32	0.034
2	2223972	rs11127372	MYT1L	Strong Candidate	0.38	0.034
6	157437279	rs12212699	ARID1B	High Confidence	2.6	0.034
7	146467409	rs199643489	CNTNAP2	Strong Candidate	2.21	0.034
12	13762597	rs2268098	GRIN2B	High Confidence	0.43	0.034
12	13763455	rs1805481	GRIN2B	High Confidence	0.43	0.034
12	13810877	rs11055566	GRIN2B	High Confidence	2.5	0.034
22	51151724	rs6010061	SHANK3	High Confidence	0.43	0.034
3	2152889	rs4300993	CNTN4	Strong Candidate	2.36	0.035
4	113914277	rs6849022	ANK2	High Confidence	2.25	0.035
5	11386206	rs1479617	CTNND2	Strong Candidate	2.11	0.035
2	51173811	rs7584445	NRXN1	Strong Candidate	0.46	0.036
5	11538379	rs6893111	CTNND2	Strong Candidate	2.2	0.036
12	14001257	rs67376488	GRIN2B	High Confidence	2.26	0.036
3	54283689	NA	CACNA2D3	Strong Candidate	0.4	0.037
6	157442069	rs17088149	ARID1B	High Confidence	2.59	0.037
12	13891551	rs2300261	GRIN2B	High Confidence	0.42	0.037
12	14077636	rs10845862	GRIN2B	High Confidence	0.3	0.037
12	14125564	rs10505778	GRIN2B	High Confidence	2.34	0.037
15	26872003	rs12907740	GABRB3	Strong Candidate	2.45	0.037
15	93559387	rs11074126	CHD2	Strong Candidate	0.43	0.037
3	2195692	rs55883839	CNTN4	Strong Candidate	0.4	0.038
7	103402243	rs10487172	RELN	Strong Candidate	0.37	0.038
12	13880012	rs12317264	GRIN2B	High Confidence	3.13	0.038
12	13960034	rs4764034	GRIN2B	High Confidence	2.65	0.038
21	38740824	rs12483205	DYRK1A	High Confidence	0.34	0.038
3	2425824	rs9830036	CNTN4	Strong Candidate	2.37	0.039
12	13972323	NA	GRIN2B	High Confidence	0.46	0.039
3	2280464	rs1178531	CNTN4	Strong Candidate	0.43	0.04
12	13948065	rs220558	GRIN2B	High Confidence	0.45	0.04
15	26865933	NA	GABRB3	Strong Candidate	2.35	0.04
18	44540376	rs12386118	KATNAL2	Strong Candidate	1.89	0.04
2	50207044	rs7608575	NRXN1	Strong Candidate	2.22	0.041
3	2498369	rs67890258	CNTN4	Strong Candidate	0.41	0.041
7	116317877	rs40239	MET	Strong Candidate	2.2	0.041
7	147205780	rs1881443	CNTNAP2	Strong Candidate	2.3	0.041
12	14005024	rs2193512	GRIN2B	High Confidence	0.47	0.041

15	26832891	rs11633705	GABRB3	Strong Candidate	2.7	0.041
15	26833912	rs7166689	GABRB3	Strong Candidate	2.7	0.041
7	145928134	rs28610113	CNTNAP2	Strong Candidate	2.45	0.042
12	13810845	rs2300235	GRIN2B	High Confidence	2.16	0.042
12	13909056	rs58125071	GRIN2B	High Confidence	3.08	0.042
15	26840998	rs1426210	GABRB3	Strong Candidate	2.38	0.042
15	26870064	rs12595837	GABRB3	Strong Candidate	2.38	0.042
15	93539390	rs144685798	CHD2	Strong Candidate	2.38	0.042
21	42158664	rs56106057	DSCAM	Strong Candidate	0.33	0.042
22	51138535	rs13054536	SHANK3	High Confidence	0.54	0.042
3	2145937	NA	CNTN4	Strong Candidate	0.27	0.043
3	2857557	NA	CNTN4	Strong Candidate	0.45	0.043
7	103521249	rs4729935	RELN	Strong Candidate	0.46	0.043
7	147630586	NA	CNTNAP2	Strong Candidate	2.06	0.043
12	13953703	rs2300270	GRIN2B	High Confidence	2.54	0.043
12	13955362	rs2300271	GRIN2B	High Confidence	2.48	0.043
12	13948760	rs220562	GRIN2B	High Confidence	0.46	0.044
12	13957167	NA	GRIN2B	High Confidence	0.46	0.044
3	20084079	rs1915922	KAT2B	Strong Candidate	2.75	0.045
11	70469410	rs4980545	SHANK2	Strong Candidate	0.37	0.045
11	70481872	rs76804246	SHANK2	Strong Candidate	0.39	0.045
12	13752832	rs2268097	GRIN2B	High Confidence	0.42	0.045
12	13753829	rs1805518	GRIN2B	High Confidence	0.42	0.045
12	13753852	rs1805519	GRIN2B	High Confidence	0.42	0.045
15	26843901	rs7178850	GABRB3	Strong Candidate	2.53	0.045
15	26846584	rs1834533	GABRB3	Strong Candidate	2.53	0.045
15	26849185	rs7172598	GABRB3	Strong Candidate	2.53	0.045
15	26849884	NA	GABRB3	Strong Candidate	2.53	0.045
2	50244128	rs10181439	NRXN1	Strong Candidate	2.04	0.046
12	14001534	rs2417306	GRIN2B	High Confidence	0.46	0.046
12	14037970	rs2041986	GRIN2B	High Confidence	2.21	0.046
21	42166769	rs2210272	DSCAM	Strong Candidate	0.5	0.046
3	20091691	NA	KAT2B	Strong Candidate	2.05	0.047
7	103468842	NA	RELN	Strong Candidate	2.02	0.047
12	13743245	rs2041889	GRIN2B	High Confidence	0.48	0.047
15	93536197	rs2272457	CHD2	Strong Candidate	0.43	0.047
3	2576058	rs11719503	CNTN4	Strong Candidate	2.33	0.048
3	2726271	rs199966573	CNTN4	Strong Candidate	0.21	0.048
4	114068306	rs6854397	ANK2	High Confidence	2.07	0.048
12	13913509	rs73053644	GRIN2B	High Confidence	2.55	0.048
12	13949973	rs220564	GRIN2B	High Confidence	0.46	0.048

12	13954737	rs220568	GRIN2B	High Confidence	0.46	0.048
12	13981051	rs7956383	GRIN2B	High Confidence	2.21	0.048
15	93484106	rs11632302	CHD2	Strong Candidate	0.43	0.048
15	93488094	NA	CHD2	Strong Candidate	0.43	0.048
3	2148393	rs2729003	CNTN4	Strong Candidate	0.49	0.049
12	13797570	rs2216215	GRIN2B	High Confidence	2.19	0.049
12	13897461	rs2268122	GRIN2B	High Confidence	0.37	0.049
12	13913336	rs17761184	GRIN2B	High Confidence	2.21	0.049

Supplementary Table 4.4. All candidate genes ranked by association with the "declining" class compared to the "normative" class, based on SNP-level results collapsed by gene.

CHR	Gene	SFARI Gene Class	SNP Count	p
2	BCL11A	Strong Candidate	20	0.002
3	CNTN4	Strong Candidate	432	0.002
3	KAT2B	Strong Candidate	48	0.002
7	CNTNAP2	Strong Candidate	446	0.002
5	CTNND2	Strong Candidate	188	0.003
15	CHD2	Strong Candidate	95	0.005
21	DYRK1A	High Confidence	16	0.006
5	RANBP17	Strong Candidate	23	0.007
2	NRXN1	Strong Candidate	177	0.008
21	DSCAM	Strong Candidate	255	0.008
12	GRIN2B	High Confidence	584	0.01
11	SHANK2	Strong Candidate	146	0.011
6	ARID1B	High Confidence	64	0.012
11	DEAF1	Strong Candidate	25	0.012
7	RELN	Strong Candidate	147	0.013
15	GABRB3	Strong Candidate	162	0.016
18	KATNAL2	Strong Candidate	53	0.018
4	ANK2	High Confidence	130	0.02
3	CACNA2D3	Strong Candidate	244	0.027
22	SHANK3	High Confidence	17	0.028
10	PTEN	High Confidence	24	0.03
2	CUL3	Strong Candidate	11	0.032
7	MET	Strong Candidate	18	0.041
7	KMT2C	Strong Candidate	15	0.055
3	FOXP1	Strong Candidate	150	0.056
6	SYNGAP1	High Confidence	3	0.062
12	GRIP1	Strong Candidate	213	0.071
2	SCN2A	High Confidence	27	0.077
3	SLC6A1	Strong Candidate	21	0.077
18	ASXL3	High Confidence	99	0.08
13	INTS6	Strong Candidate	13	0.081
14	IRF2BPL	Strong Candidate	2	0.083
16	ANKRD11	Strong Candidate	59	0.094
16	CACNA1H	Strong Candidate	16	0.132
1	ASH1L	High Confidence	20	0.15
16	USP7	Strong Candidate	15	0.17
10	WAC	Strong Candidate	92	0.218

2	SPAST	Strong Candidate	8	0.231
1	POGZ	High Confidence	3	0.24
3	SETD5	High Confidence	12	0.282
12	MED13L	Strong Candidate	9	0.286
2	TBR1	High Confidence	2	0.318
1	KDM5B	Strong Candidate	39	0.349
11	KMT2A	Strong Candidate	8	0.418
20	ADNP	High Confidence	4	0.655
1	ILF2	Strong Candidate	4	0.704

CHAPTER 5:

DISCUSSION

5.1. Summary of findings

ASD is a neurodevelopmental disorder that is heterogeneous in presentation and etiology. Comorbid conditions, symptom profiles, and variable onset trajectories contribute to phenotypic heterogeneity, while genetic and early-life environmental risk factors contribute to etiologic heterogeneity (Gardener, Spiegelman, & Buka, 2009; Landa, Gross, Stuart, & Faherty, 2013; Landa, Holman, & Garrett-Mayer, 2007; Sandin et al., 2014; Schieve et al., 2012; Veatch, Veenstra-Vanderweele, Potter, Pericak-Vance, & Haines, 2014). The goal of this dissertation was to examine comorbidity, environmental, and genetic risk factors associated with ASD in the context of other neurodevelopmental disorders and general early neurodevelopment.

Results from this dissertation add to the evidence for increased risk of other neurodevelopmental disorders and physical comorbidity among ASD (Bauman, 2010; Gurney, McPheeters, & Davis, 2006; Matson & Goldin, 2013; Schieve et al., 2012). Using data from a nationally representative population-based survey (NCS-A), we found that 70% of adolescents with parent reported ASD had concurrent LD and 42% had concurrent ADHD. Additionally, we found that ASD adolescents were at increased risk for gastrointestinal problems (OR = 5.60, $p = 0.004$), epilepsy and seizures (OR = 9.97, $p < 0.001$), allergies (OR = 3.48, $p = 0.004$), acne (OR = 3.25, $p=0.020$), heart problems (OR= 4.08, $p = 0.020$), and early morning awakening (OR = 2.44, $p = 0.04$). Of note, we see that adolescents with LD were also at increased risk for ADHD (OR = 6.13, $p < 0.001$), gastrointestinal problems (OR = 1.70, $p = 0.030$), and epilepsy/seizure (OR = 2.82, $p = 0.001$), suggesting an association with neurodevelopmental disorders in general. This is consistent with previous literature also showing associations for these disorders

across ID and other types of developmental delay, where effect sizes are larger with more severe neurodevelopmental disorders (Schieve et al., 2012).

However, as the first population-based based study to evaluate mental health conditions among ASD by direct evaluation with the subject, we do not confirm previous findings of increased risk for depression and anxiety among individuals with ASD from clinical studies and parent report surveys (Gurney et al., 2006; Joshi et al., 2013; McPheeters, Davis, Navarre, & Scott, 2011). One potential reason for this could be that previous studies ascertained clinically could be biased toward a more severely affected treatment-seeking population of individuals with ASD. Further, other population based studies assessing mental disorders among ASD have relied on parent report, which may not be accurate for assessing the presence of internalizing disorders such as depression and anxiety (De Los Reyes et al., 2015). However, another explanation for this could be that we evaluated mental disorders using the CIDI (Robins et al.), which is not validated for use among individuals with ASD. Therefore, it might not be the appropriate measurement tool for these disorders, as the sensitivity for evaluation of these disorders in ASD is unknown.

Results from this dissertation also provide further support for the hypothesis that the pre/perinatal period is a critical window of risk for non-genetic factors that may impact development of ASD (Gardener et al., 2009; Gardener, Spiegelman, & Buka, 2011; Schieve, Clayton, Durkin, Wingate, & Drews-Botsch, 2015). Using nationally representative survey data (NCS-A), we found prenatal exposure to alcohol (ASD: OR = 2.43, p = 0.017, LD: OR = 1.42, p = 0.039), maternal urinary tract infection during pregnancy (ASD: OR=4.46, p=0.018; LD: OR=1.67, p=0.036), slow heartbeat at birth

(ASD: OR=6.27, p=0.028; LD: OR=1.86, p=0.031), respiratory distress at birth (ASD: OR=5.85, p=0.020; LD: OR=2.28, p=0.003, and preterm birth (ASD; OR=3.57, p=0.018; LD: OR=2.33, p=0.001) to be associated with both ASD and LD. This is in support of other research suggesting that prenatal risk factors contribute to multiple neurodevelopmental outcomes (Krakowiak et al., 2012; Schieve et al., 2010; Schieve et al., 2015; Schieve et al., 2016; Tamaru et al., 2011). However, after controlling for preterm birth, associations between neonatal conditions and LD dissipated while remaining significant for ASD (convulsions: OR=9.23, p=0.008); jaundice: OR=3.21, p=0.028; requirement of oxygen: OR=2.26, p=0.048; blood transfusion: OR=7.04, p=0.018). Of note, persistent proteinuria was associated with ASD (OR = 13.13, p = 0.005), but not LD and was the only obstetric complication examined in our sample, including gestational diabetes, that was associated with either neurodevelopmental disorder. Previous research has been inconsistent in results linking gestational diabetes to ASD (Gardener et al., 2009; Guinchat et al., 2012; Krakowiak et al., 2012); however, preeclampsia and related symptoms, such as proteinuria, have been far more consistently linked to ASD (Gardener et al., 2009; Polo-Kantola et al., 2014; Walker et al., 2015; Wallace, Anderson, & Dubrow, 2008). As gestational diabetes increases the mother's risk for developing preeclampsia, it is possible that the consequences of preeclampsia, such as increased inflammation, vascular damage, decreased placental perfusion, and decreased blood flow and oxygen to the fetus, are more closely involved in the mechanisms contributing to ASD risk rather than those specifically related to gestational diabetes. Further work is needed to understand the possible mechanisms liking this finding to ASD.

There may be clinical implications of these findings regarding comorbid conditions and prenatal risk factors for ASD and LD. First, treating sleep and physical health problem in kids with ASD could improve not only quality of life, but also behavioral symptoms associated with ASD (Bauman, 2010). Therefore, greater awareness of comorbid conditions affecting adolescents with ASD and LD could improve quality of life and school performance, especially for those with LD, where services provided are primarily through the educational system, rather than clinical care. Several perinatal risk factors associated with ASD, such as proteinuria, birth outcomes, and neonatal complications may warrant timely developmental surveillance which could lead to earlier intervention, and therefore, better prognosis. However, further work should be done in appropriate samples to try to disentangle the pathways leading to compilations in neurodevelopment to know when and how to intervene.

In our examination of early cognitive development among infants at elevated risk for developing ASD, we found three distinct subpopulations of early cognitive and motor development trajectories which we named "normative", "intermediate", and "declining" based on patterns of MSEL domain scores over time. Nearly half of the "declining" class consisted of ASD cases, compared to 15% of the intermediate class, and 2% of the normative class. While cognitive developmental trajectory was associated with ASD, it is not a direct surrogate of ASD.

The declining class was marginally associated with a liberally defined burden score of common genetic variation associated with ASD (OR = 1.66, $p = 0.083$). A candidate gene approach assessing independent SNPs from 47 ASD-implicated genes yielded several nominally ($p < 0.05$) significant findings associated with trajectory class

membership. The two top ranked genes associated with the trajectory classes were *CNTN4* and *CNTNAP2*. These genes both code for proteins involved neuronal cell adhesion and migration (Rodenas-Cuadrado, Ho, & Vernes, 2014; Shimoda & Watanabe, 2009). Further assessment of unpruned SNPs in *CNTN4* on chromosome 3 revealed an association peak of suggestive significance below rs4075530 for the "intermediate" class compared to the "normative" class. There have been no other associations reported in this linkage disequilibrium block in the NHGRI GWAS catalog; however, several genome-wide significant associations for intelligence traits in a ADHD sample have been identified in the same gene nearby approximately 50kb downstream (Loo et al., 2012). Regional mapping of unpruned SNPs in *CNTNAP2* revealed an association peak for the "declining" class at the 5' end of the gene. This peak lies under previous findings implicating risk for bipolar disorder, schizophrenia, and late-onset Alzheimer's disease (Hirano et al., 2015; Wang, Liu, & Aragam, 2010). Association peaks from our results in either gene generally did not align with association peaks from the Psychiatric Genomics Consortium (PGC).

The use of a phenotype associated with underlying ASD traits, rather than diagnostic criteria gives some additional insight into potential underlying biology. Interestingly, previous findings ASD case-control studies reporting on *CNTN4* largely implicate the role of rare CNVs, rather than common SNPs (Cottrell et al., 2011). Here, we see SNP associations using a phenotype that is associated with ASD, rather than diagnostic criteria. It could be that common variants of *CNTN4* affect neurodevelopment, but to a lesser extent than gene disrupting structural variants that have been so strongly implicated in ASD case status. Additionally, the use of these phenotypes yielded p-values for SNP

associations in these genes similar in magnitude to results from PGC (n=10,610) where the sample size was over 50 times the size of ours (n=198). Further investigation into other candidate genes should be done to investigate this pattern.

5.2. Summary of strengths and challenges

The two study designs used in this dissertation offered both strengths and challenges. The NCS-A, a nationally representative population-based sample offers representativeness not typical in a clinically selected sample. The estimates we report accurately represent that of the target population – US adolescents attending school during the time of data collection (2001-2004). However, we face a tradeoff between representativeness and rigorous phenotyping. While the NCS-A is the only US representative sample that has collected face-to-face diagnostic interviews on a range of mental disorders affecting adolescents (i.e. depression, bipolar disorder, anxiety, eating disorders, ADHD, etc.), specific diagnoses including ASD, LD, physical health conditions, and prenatal and perinatal factors were collected via parent report. Therefore, we are less confident in the accuracy of these variables due to the potential for recall bias for health conditions and prenatal/perinatal factors and unconfirmed diagnoses for ASD and LD.

Furthermore, while the overall NCS-A sample size used in this dissertation was quite large (n=6,296), the number of adolescents in the ASD-affected analytic group was small (n=51) due to the low prevalence of ASD at the time of data collection (~ 0.08%) (CDC, 2007). This resulted in large standard errors in prevalence and association estimates. While a case-control study design would have yielded a larger ASD-affected

group, it is likely that our prevalence estimates of comorbid conditions would have been elevated due to ascertainment bias.

Similarly, we dealt with relatively small sample sizes in EARLI/IBIS, enriched risk cohorts, compared to what is typically attainable through case-control designs. However, the use of enriched risk longitudinal design allows for prospective data collection, in-depth phenotyping, and biomarker collection. Due to the careful phenotypic, biologic, and risk factor characterization of these participants, obtaining large sample sizes on the order of case-control designs is cost prohibitive. While not representative of the general population, an enriched risk sample yields a higher proportion of ASD affected individuals compared to sampling from the general population, making outcome numbers feasible for association analyses while preserving the benefits of a prospective design.

Additionally, the work on genetic association with developmental trajectory is limited by a small sample size to date, so it is unclear whether this phenotyping approach will yield more insights than traditional case-control designs until it can be compared with comparable sample sizes. However, given the magnitude of statistical significance achieved in our modest sample size (n=198) for the genetic association analyses compared to the largest known ASD case-control GWAS to date (n=10,610), this approach seems promising. For this project our sample also consists of individuals from a variety of ethnicities. The modest sample size precluded us from stratifying analyses by ancestral background. While we did adjust analyses for principal components of ancestry, there is potential for residual confounding by population stratification.

5.3. Future directions

The work presented in this dissertation can be expanded upon to gain additional insight into comorbidity, environmental, and genetic risk associated with ASD and other neurodevelopmental disorders. First, to assess true specificity for comorbid conditions and early life risk factors associated with ASD compared to other neurodevelopmental disorders, such as LD, it would be advantageous to directly calculate change in risk for ASD compared to LD instead of a typically developing group.

Second, prevalence and association estimates of individual comorbid conditions do not reveal the extent to which the same cases may have multiple comorbid conditions. While many conditions are associated, it would be informative to know if particular comorbid conditions tend to occur together in the same individuals, while other sets of conditions co-occur in other individuals with neurodevelopmental disorders. To do this, one could use latent class analysis, or other cluster analysis methods that recognize patterns among variables. Others have taken a similar approach mining electronic health record data (Doshi-Velez, Ge, & Kohane, 2014), but this could be informative among a representative sample such as the NCS-A. Given the strong overlap between many of the common comorbid conditions across neurodevelopmental disorders, this would better direct us toward etiologic subtypes of disease. A similar clustering approach should be used for assessing prenatal and perinatal risk factors associated with ASD. A review of over 85 studies examining prenatal and perinatal risk factors for ASD concluded that, in light of so many implicated risk factors and inconsistency among findings, future work should investigate combinations of risk factors that may provide better insight into the

pathways and mechanisms by which these early-life factors are associated (Guinchat et al., 2012).

The polygenic risk score (PRS) analyses may be limited by lack of ASD-associated SNPs that inform the score calculation. Increasing sample size of the PGC discovery sample could improve ability to identify common variants associated with ASD, as was achieved in their schizophrenia case-control GWAS (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). As these analyses are currently underway by the PGC, we plan to re-calculate risk scores in our sample when they become publically available. Additionally, PRS and candidate gene analyses presented in this dissertation will benefit from obtaining genotype data for the IBIS participants included in the latent trajectory model. Genotyping for these participants is currently underway, which will approximately double the sample size for the genetic association portion of this project.

The combination of EARLI and IBIS will still leave us with a small sample size by traditional genome-wide association standards, and thus, still lack power to reach genome-wide significant SNP associations. Future extensions of this work in larger cohorts would benefit from an agnostic genome-wide investigation of early cognitive and motor development. With power for genome-wide discovery, one could evaluate the overlap between pathways and gene-sets associated with ASD diagnostic category and developmental trajectory prior to diagnosis to assess whether these phenotypic manifestations are operating similarly on a molecular level. If new gene-sets are implicated, this could help disentangle some of the genetic heterogeneity contributing to

ASD by identifying loci affecting general neurodevelopmental traits rather than ASD specifically.

5.4. Public health significance

Population-based studies suggest the incidence of ASD is on the rise (Boyle et al., 2011). As such, it is important to understand the constellation of physical and mental health problems that will affect an aging population with ASD that is also increasing in size. This dissertation used nationally representative data to support previous work indicating that children with ASD and other neurodevelopmental disorders are at increased risk for developing a number of physical health problems. These conditions may significantly decrease quality of life if not properly identified and treated. Awareness of these conditions by clinicians treating individuals with neurodevelopmental disorders could aid in earlier recognition of potential physical and mental health problems that may be contributing to behavioral symptoms, or decreased performance in school or work (Bauman, 2010).

There is substantial evidence for contribution of both genetic and environmental elements to ASD risk (Hallmayer et al., 2011; Sandin et al., 2014) and that the critical window of risk for exposure to non-genetic risk factors lies in the pre-conception to neonatal period of life (Bauman & Kemper, 2005; Newschaffer, Fallin, & Lee, 2002). Therefore, depending on the child's genetic liability for ASD, identification of malleable non-genetic risk factors could potentially prevent or decrease severity of some cases. While more work is needed to understand exact combinations, timing, and mechanisms of prenatal and perinatal risk factors contributing to ASD risk, results from this

dissertation substantiate previous implications of several factors, including those associated with preeclampsia, from a representative non-clinical sample.

Although more work is needed to characterize the genetic underpinnings of ASD, understanding the genetic architecture can lead to better risk prediction, which can lead to earlier intervention and better prognosis. Additionally, knowledge of genes involved will give insight into biological pathways involved in disease, which can potentially lead to development of new treatments. Similarly, increased knowledge of genetic underpinnings of a child's developmental trajectory may allow us to eventually screen children who are more susceptible to decline and target them for early intervention, regardless of their risk for ASD.

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